527 Rec'd PCT/PTO 12 DEC 2000

FORM PTO-1390 (REV. 10-2000) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY'S DOCKET NUMBER 20251P
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		U.S. APPLICATION NO. (If known, see 37 CFR 1.5) 09/719485
INTERNATIONAL APPLICATION NO. PCT/US99/12773	INTERNATIONAL FILING DATE June 8, 1999	PRIORITY DATE CLAIMED June 12, 1998

TITLE OF INVENTION
CLONING AND IDENTIFICATION OF THE MOTILIN RECEPTOR

APPLICANT(S) FOR DO/EO/US
Scott D. Feighner, Arthur A. Patchett, Carina Tan, Karen McKee, Douglas MacNeil, Andrew D. Howard, Sheng-Shung Pong

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures [35 U.S.C. 371(f)] at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☐ A proper Demand for International Preliminary Examination was made and the US was elected by the expiration of the 19th month from the earliest claimed priority date (PCT Article 31).
5. ☒ A copy of the International Application as filed [35 U.S.C. 371(c)(2)].
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☐ has been communicated by the International Bureau.
   c. ☒ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed [35 U.S.C. 371(c)(2)].
7. ☒ Amendments to the claims of the International Application under PCT Article 19 [35 U.S.C. 371(c)(3)].
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 [35 U.S.C. 371(c)(3)].
9. ☐ An oath or declaration of the inventor(s) [35 U.S.C. 371(c)(4)].
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 [35 U.S.C. 371(c)(5)].

Items 11 to 16 below concern other document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☐ A **FIRST** preliminary amendment.
    ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☐ Other items or information:

EXPRESS MAIL CERTIFICATE
DATE OF DEPOSIT December 12, 2000
EXPRESS MAIL NO EL 520857913US
I HEREBY CERTIFY THAT THIS CORRESPONDENCE IS BEING DEPOSITED WITH THE UNITED STATES POSTAL SERVICE AS EXPRESS MAIL "POST OFFICE TO ADDRESSEE" BEFORE 5 P.M. ON THE ABOVE DATE IN AN ENVELOPE ADDRESSED TO ASSISTANT COMMISSIONER FOR PATENTS, WASHINGTON, D.C. 20231.
MAILED BY B. Crowley
DATE 12/12/00

528 Rec'd PCT/PTO 12 DEC 2000

U.S. APPLICATION NO (If known, see 37 CFR 1 5) 09/719485	INTERNATIONAL APPLICATION NO PCT/US99/12773	ATTORNEY'S DOCKET NUMBER 20251P

	CALCULATIONS	PTO USE ONLY
17. [X] The following fees are submitted: **BASIC NATIONAL FEE [37 CFR 1.492(a)(1)-(5)]:** Neither international preliminary examination fee (37 CFR 1.482) nor international search fee [37 CFR 1.445(a)(2)] paid to USPTO and International Search Report not prepared by the EPO or JPO ..... **$1,000.00** International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO ..... **$860.00** International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee [37 CFR 1.445(a)(2)] paid to USPTO ..... **$710.00** International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) ..... **$690.00** International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) **$100.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$690.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than [ ] 20 [ ] 30 months from the earliest claimed priority date [37 CFR 1.492(e)].	$0.00	

Claims	Number Filed	Number Extra	Rate	CALCULATIONS	PTO USE ONLY
Total Claims	8 - 20 =	0	X **$18.00**	$0.00	
Independent Claims	2 - 3 =	0	X **$80.00**	$0.00	
Multiple dependent claim(s) (if applicable)			+ **$270.00**	$0.00	
TOTAL OF ABOVE CALCULATIONS =				$690.00	
[ ] Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.					
SUBTOTAL =				$690.00	
Processing fee of **$130.00** for furnishing the English translation later than [ ] 20 [ ] 30 months from the earliest claimed priority date [37 CFR 1.492(f)]. +				$0.00	
TOTAL NATIONAL FEE =				$690.00	
Fee for recording the enclosed assignment [37 CFR 1.21(h)]. The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property. +					
TOTAL FEES ENCLOSED =				$690.00	
				Amount to be refunded	
				charged	

a. [ ] A check in the amount of $ __________ to cover the above fees is enclosed.

b. [X] Please charge my Deposit Account No. 13-2755 in the amount of $690.00 to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to the Deposit Account No. 13-2755. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive [37 CFR 1.137(a) or (b)] must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

MERCK & CO., INC.
Patent Department, RY60-30
P.O. Box 2000
126 East Lincoln Avenue
Rahway, New Jersey 07065-0970

DATE: December 12, 2000

PHONE #: (732) 594-1273

SIGNATURE

Anna L. Cocuzzo
NAME

42,452
REGISTRATION NUMBER

Computer generated form "TransLetter (DO-EO-US)" (PCT Folder), Merck & Co., Inc., 10/3/00

TITLE OF THE INVENTION
CLONING AND IDENTIFICATION OF THE MOTILIN RECEPTOR

CROSS-REFERENCE TO RELATED APPLICATIONS

xxxxx

STATEMENT REGARDING FEDERALLY-SPONSORED R&D

xxxxx

REFERENCE TO MICROFICHE APPENDIX

xxxxx

FIELD OF THE INVENTION

The present invention is directed to a novel human DNA sequence encoding a motilin receptor, the receptor encoded by the DNA, and the uses thereof.

BACKGROUND OF THE INVENTION

Gastrointestinal (GI) motility is a coordinated neuromuscular process which transports nutrients through the digestive system. Impaired GI motility, can lead to irritable bowel syndrome, constipation and diabetic and post-surgical gastroporesis and is one of the largest health care burdens of industrialized nations. Motilin, a 22 amino acid prokinetic peptide is expressed throughout the gastrointestinal tract in a number of species including humans. Released from endochromafffin cells of the small intestine, motilin exerts a profound effect on gastric motility with the induction of interdigestive (phase III) antrum and duodenal contractions. The unrelated macrolide antibiotic erythromycin also possesses prokinetic properties mediated by its interaction with motilin receptors. These account for erythromycin's GI side-effects, including vomiting, nausea, diarrhea and abdominal muscular discomfort.

Motilin receptors have been detected in the GI tract and recently in the central nervous system, but their molecular structure has not been reported. Although motilin receptor characterization has been actively pursued in humans and other species since the isolation of motilin from

porcine intestine in 1972, the receptor itself has not been isolated nor cloned.

Motilin is highly conserved across species and is synthesized as part of larger pre-prohormone. Mature 22 amino acid motilin is generated by removal of its secretory signal peptide and cleavage at the first C-terminally located dibasic prohormone convertase recognition site. Using radioligand binding, autoradiography and *in vitro* biossays, high affinity and low density, motilin receptors were detected in smooth muscle cells of the gastrointestinal tract of humans, cats and rabbits. Cerebellar brain receptors for motilin were also described supporting the notion that motilin may act in the central nervous system. Native motilin receptors appear to be coupled to G proteins and activate the phosphlipase C signal tranduction pathway resulting in Ca^{2+} influx through L-type channels.

The development of safe and selective motilin receptor agonists is likely to aid the treatment of disorders resulting from impaired GI motility. Thus, it would be desirable to be able to isolate, and clone the motilin receptor, and to use this in assays for agonists and antagonists.

SUMMARY OF THE INVENTION

The present invention is directed to a novel G-protein coupled receptor (GPCR), designated as motilin receptor. Two spliced forms of the motilin receptor were identified: MTL-R1A, which encodes a functional seven-transmembrane domain form, and MTL-R1B, which encodes a truncated five-transmembrane domain form. Both forms make up embodiments of this invention.

Another aspect of this invention are nucleic acids which encode the motilin receptor, which are isolated, or free from associated nucleic acids.

Other aspects of this invention include assays for identifying motilin ligands which are agonists and antagonists of a motilin receptor comprising contacting a candidate ligand with a motilin receptor and determining if binding occurred.

Another aspect of this invention is a method for determining whether a ligand is capable of binding to a motilin receptor comprising:

(a) transfecting test cells with an expression vector encoding motilin receptor;

(b) exposing the test cells to the ligand;

(c) measuring the amount of binding of the ligand to the motilin receptor;

(d) comparing the amount of binding of the ligand to the motilin receptor in the test cells with the amount of binding of the ligand to control cells that have not been transfected with the motilin receptor

where if the amount of binding of the ligand to the test cells is greater than the amount of binding of the ligand to the control cells, then the substance is capable of binding to motilin receptor.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the DNA sequence of motilin receptor gene including 5' untranslated region (SEQ.ID.NO.:1). Intronic sequences are shown in lower case type.

Figure 2 shows the DNA sequence of motilin receptor spliced form A (MTL-R1A) (SEQ.ID.NO.:2).

Figure 3 shows deduced amino acid sequence of MTL-R1A (SEQ.ID.NO.:3).

Figure 4 shows the DNA sequence of motilin receptor spliced form B (MTL-R1B) (SEQ.ID.NO.:4).

Figure 5 shows the deduced amino acid sequence of MTL-R1B (SEQ.ID.NO.:5).

Figures 6 A-C compare DNA and protein sequence for MTL-R1A and MTL-R1B.

Figure 7 shows the DNA sequence of puffer fish clone 75E7 (SEQ.ID.NO.:6).

Figure 8 shows the deduced amino acid sequence of puffer fish clone 75E7 protein sequences (SEQ.ID.NO.:7).

Figure 9 shows the comparison of human MTL-R1A and puffer fish clone 75E7 protein sequences.

Figure 10 is a graph illustrating the pharmacological characterization of the cloned MTL-R1A in the aequorin bioluminescence assay in HEK-293 cells.

Figure 11 is a graph illustrating the pharmacological characterization of the cloned MTL-R1A in the [^{125}I]-Tyr^7-human motilin binding assay.

As used throughout the specification and claims, the following definitions apply:

"Substantially free from other proteins" means at least 90%, preferably 95%, more preferably 99%, and even more preferably 99.9%, free of other proteins. Thus, for example, a MTL-R1 protein preparation that is substantially free from other proteins will contain, as a percent of its total protein, no more than 10%, preferably no more than 5%, more preferably no more than 1%, and even more preferably no more than 0.1%, of non- MTL-R1 proteins. Whether a given MTL-R1 protein preparation is substantially free from other proteins can be determined by such conventional techniques of assessing protein purity as, *e.g.*, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) combined with appropriate detection methods, *e.g.*, silver staining or immunoblotting.

"Substantially free from other nucleic acids" means at least 90%, preferably 95%, more preferably 99%, and even more preferably 99.9%, free of other nucleic acids. Thus, for example, a MTL-R1 DNA preparation that is substantially free from other nucleic acids will contain, as a percent of its total nucleic acid, no more than 10%, preferably no more than 5%, more preferably no more than 1%, and even more preferably no more than 0.1%, of non- MTL-R1 nucleic acids. Whether a given MTL-R1 DNA preparation is substantially free from other nucleic acids can be determined by such conventional techniques of assessing nucleic acid purity as, *e.g.*, agarose gel electrophoresis combined with appropriate staining methods, *e.g.*, ethidium bromide staining, or by sequencing.

"Functional equivalent" means a receptor which does not have the exact same amino acid sequence of a naturally occurring motilin receptor, due to alternative splicing, deletions, mutations, or additions, but retains at least 1%, preferably 10%, and more preferably 25% of the biological activity of the naturally occurring receptor. Such derivatives will have a significant homology with a motilin receptor and

can be detected by reduced stringency hybridization with a DNA sequence obtained from a motilin receptor. The nucleic acid encoding a functional equivalent has at least about 50% homology at the nucleotide level to a naturally occurring receptor nucleic acid.

"Ligand" means any molecule which binds to a motilin receptor of this invention. These ligands can have either agonist, partial agonist, partial antagonist or antagonist activity.

DETAILED DESCRIPTION OF THE INVENTION

The cloning of GPCR's related to the hypothalamic and pituitary receptor for the growth hormone (GH) secretagogues (GHSs) which mediate sustained pulsatile GH release has been recently described. (McKee *et. al.*, 1997 *Genomics 46*:426-434, which is hereby incorporated by reference). One of these clones, GPR38, possessed the most significant amino acid sequence identity to the human GHSR (52%) (rising to as high as 86% in transmembrane domains (TM). GPR38 was classified as an orphan GPCR (GPCRs for which a natural ligand has not been identified).

GPR38 was isolated from a human genomic DNA library and contained a single intron of approximately 1 kb, as shown in FIGURE 1. cDNA clones were isolated to obtain the nucleotide sequence of correctly spliced GPR38 mRNA. Efforts to isolate cDNA clones by standard library screening proved unsuccessful.

A combination of RACE and RT-PCR techniques resulted in the identification of two spliced forms for GPR38. These two GPR38 cDNAs use distinct splice donor sites and a common acceptor site (perfect match to consensus exon-intron splice acceptor junction sequence [pyrimidine-rich stretch ag/TG]). GPR38-A mRNA (imperfect match to consensus donor sequence [TGC/gt]) encodes a polypeptide of 412 amino acids with seven alphahelical TM domains, the hallmark feature of GPC-Rs, whereas GPR38-B encodes a 363 amino acid polypeptide with five TM domains (perfect donor sequence [CCG/gt]). Northern blot analysis failed to reveal an expression profile for GPR38. However, when RNase protection was employed expression was demonstrated in stomach, thyroid and bone marrow.

It accordance with this invention, it has been found that GPR38 is the motilin receptor. Thus, this invention is directed to the human motilin receptor, its functional equivalents, motilin receptors from other species which can be isolated using fragments of the human motilin DNA as probes, and to splice varients of the motilin receptor.

The intact motilin receptor of this invention was found to have structural features which are typical of G-protein linked receptors, including seven transmembrane (TM) domains, three intra- and extracellular loops, and the GPCR protein signature sequence. The TM domains and GPCR protein signature sequence are noted in the protein sequences of the GPCR in Figures 6A-C.

A high-throughput assay was developed which measures Ca^{2+} realease with the bioluminescent Ca^{2+} sensitive-aequorin reporter protein (capable of measuring ligand-induced IP3-coupled mobilization of intracellular calcium and concomitant calcium-induced aequorin bioluminescence). Expression of cloned GPR38-A in cell membranes was confirmed using epitope-tagged protein which revealed a single protein species with a molecular weight of approximately 45,000 daltons containing an open reading frame encoding 412 amino acids (SEQ. ID.NO.:3). The DNA and deduced amino acid sequence are given in SEQ.ID. NO.:2 and SEQ.ID. NO.:3, respectively.

A broad set of peptide and non-peptide molecules were tested at a single concentration in transiently transfected HEK-293/aeq17 cells (100 nM peptide, 10 μM non-peptide). Significant bioluminescent responses were recorded for the peptide motilin and the non-peptide macrolide erythromycin, which was reported to be a competitive agonist at motilin receptors. Full dose-response curves confirmed this observation.

Nucleotide sequence analysis revealed two splice forms of human motilin receptor both of which make up further aspects of this invention. The first (MTL-R1A) encodes a seven transmembrane domain receptor. The full length open reading frame appears to contain 412 amino acids. The second splice form (MTL-R1B) diverges in its nucleotide sequence from MTL-R1A just before the predicted amino acid of the sixth transmembrane domain (TM6).

In the MTL-R1B, TM5 is truncated and fused to a contiguous reading frame of about 86 amino acids, followed by a translation stop codon. The DNA and amino acids sequences encoding MTL-R1A and MTL-R1B are given in FIGURES 2-5.

A further aspect of this invention is a related motilin receptor gene, evident in the teleost puffer fish *Spheroides nephelus*. Screening of a puffer fish genomic library identified a single clone (75E7) containing an open reading frame of 363 amino acids (approximately 54% identical at the protein level) which contains a similar exon-intron structure to GPR38. Analysis of clone 75E7 shows an amino acid sequence to contain 363 amino acids with a molecular weight of approximately 41,323 daltons. (FIGURE 8). DNA sequence of puffer fish clone 75E7 is given in SEQ.ID.NO.:6, and a comparison of human MTL-R1A and puffer fish clone 75E7 protein sequences is given in FIGURE 9.

Another aspect of this invention relates to vectors which comprise nucleic acids encoding a motilin receptor or a functional equivalent. These vectors may be comprised of DNA or RNA; for most clôning purposes DNA vectors are preferred. Typical vectors include plasmids, modified viruses, bacteriophage and cosmids, yeast artificial chromosomes and other forms of episomal or integrated DNA that encode a motilin receptor. It is well within the skill of the ordinary artisan to determine an appropriate vector for a particular gene transfer or other use.

A further aspect of this invention are host cells which are transformed with a gene which encodes a motilin receptor or a functional equivalent. The host cell may or may not naturally express a motilin receptor on the cell membrane. Preferrably, once transformed, the host cells are able to express the motilin receptor or a functional equivalent on the cell membrane. Depending on the host cell, it may be desirable to adapt the DNA so that particular codons are used in order to optimize expression. Such adaptations are known in the art, and these nucleic acids are also included within the scope of this invention. Generally mammalian cell lines, such as HEK-293, COS , CHO, HeLa, NS/), CV-1, GC, GH3 or VERO cells are preferred host cells, but other

cells and cell lines such as *Xenopus oocytes* or insect cells, may also be used.

Human embryonic kidney (HEK 293) cells and Chinese hamster ovary (CHO) cells are particularly suitable for expression of motilin receptor proteins because these cells express a large number of G-proteins. Thus, it is likely that at least one of these G-proteins will be able to functionally couple the signal generated by interaction of motilin receptors and their ligands, thus transmitting this signal to downstream effectors, eventually resulting in a measurable change in some assayable component, *e.g.*, cAMP level, expression of a reporter gene, hydrolysis of inositol lipids, or intracellular Ca^{2+} levels.

A variety of mammalian expression vectors can be used to express recombinant motilin in mammalian cells. Commercially available mammalian expression vectors which are suitable include, but are not limited to, pCR2.2 (Invitrogen), pMClneo (Stratagene), pSG5 (Stratagene), pcDNAI and pcDNAIamp, pcDNA3, pcDNA3.1, pCR3.1 (Invitrogen), EBO-pSV2-neo (ATCC 37593), pBPV-1(8-2) (ATCC 37110), pdBPV-MMTneo(342-12) (ATCC 37224), pRSVgpt (ATCC 37199), pRSVneo (ATCC 37198), and pSV2-dhfr (ATCC 37146). Following expression in recombinant cells, motilin receptors can be purified by conventional techniques to a level that is substantially free from other proteins.

The specificity of binding of compounds showing affinity for motilin receptors is shown by measuring the affinity of the compounds for recombinant cells expressing the cloned receptor or for membranes from these cells. Expression of the cloned receptor and screening for compounds that bind to motilin receptors or that inhibit the binding of a known, radiolabeled ligand of motilin receptors to these cells, or membranes prepared from these cells, provides an effective method for the rapid selection of compounds with high affinity for a motilin receptor. Such ligands need not necessarily be radiolabeled but can also be nonisotopic compounds that can be used to displace bound radiolabeled compounds or that can be used as activators in functional assays. Compounds identified by the above method are likely to be agonists or antagonists of

motilin receptors and may be peptides, proteins, or non-proteinaceous organic molecules.

Such molecules are useful in treating a variety of gastric conditions, including gastric motility disorders (intrinsic myopathies or neuropathy), functional defects, disorders which are secondary to neurologic disorders including spinal cord transections, amyloidosis, collagen vascular disease (e.g. scleroderma), paraneoplastic syndromes, radiation-induced dysmotility, diabetes, infections, stress-related motiliy disorders, psychgenic/functional disorders, other drugs which affect motility (e.g. beta andrenergic drugs which may delay gastric emptying, cholinergic agents or opiates, or serotonin receptor antagonists), gastroparesis (diabetic or post-surgical), gastro-esophageal reflux disease, constipation, chronic idiopathis pseudo-obstruction and acute fecal impaction, postoperative ileus, gallstones, infantile collic, preparation for colonoscopy and endoscopy, duodenal intubation , irritable bowel syndrome, non-ulcer dyspepsion, non-cardiac chest pain and diarrhea.

The pharmacological characterization of the cloned MTL-R1A in the aequorin bioluminescence assay in HEK-293 cells is shown in Figure 10 and in the [^{125}I]-Tyr^7-human motilin binding assay (Figure 11). Motilin at concentrations as high as 10 μM gave no bioluminescent response above background levels in cells that were not transfected with the MTL-R1A cDNA expression vector. Similarly, non-transfected cells did not show appreciable binding of [^{125}I]-Tyr^7-human motilin.

The rank order of potency for motilin, motilin peptide fragments and non-peptide molecules is consistent with experiments performed on native motilin receptors, from stomach or intestinal tissues.

Due to the high degree of homology to GPCRs, the motilin receptor of this invention is believed to function similarly to GPCRs and have similar biological activity. They are useful in understanding the biological and physiological pathways involved in gastrointestinal motility. They may be also used to scan for motilin agonists and antagonists; as in particular to test the specificity of identified ligands.

The following, non-limiting Examples are presented to better illustrate the invention.

EXAMPLE1

Sequence Comparison of MTL-R1 (GPR38) to human GHS-R, Puffer Fish 75E7 and Identification of Alternatively Spliced Forms.

Inspection of the MTL-1 genomic DNA sequence revealed two potential mRNA splice sites corresponding to consensus boundaries for exon/intron junctions. An imperfect donor site (TGC/gt) was found at nucleotides 1929-31 (Fig. 1), a perfect donor site (CCG/gt) was found at nucleotides 2080-82, and a single perfect splice acceptor site (sequence [pyrimidine-rich stretch ag/TG]) was observed at nucleotides 2729-32. To determine which splice forms exist naturally, RACE (rapid amplification of cDNA ends) was performed on thyroid poly (A)+ mRNA and RT-PCR (reverse transcriptase polymerase chain reaction) was conducted on HEK-293/aeq17 cells transfected with the MTL-1 genomic DNA construct. Directional RACE reactions were conducted on thyroid poly (A)+ mRNA that had previously been shown by RNase protection assay to contain transcripts for MTL-1R. Primer AP1 5'-CCA TCC TAA TAC GAC TCA CTA TAG GGC-3' (SEQ.ID.NO.:8) corresponds to the 5' end of the coding region including the presumptive Met initiation codon located within the cloning vector. 5'RACE1 corresponds to the 3' end of the MTL-1R coding region including the translation termination codon TAA. 5' RACE1: 5'-TTA TCC CAT CGT CTT CAC GTT AGC GCT TGT CTC-3' (SEQ.ID.NO.:9).

RACE reactions were carried out on 1 μg of thyroid poly (A)+ mRNA using the Marathon cDNA amplification/advantage PCR kit as per the manufacturer's instructions (Clontech) using the following Touchdown PCR amplification conditions: 94°C for 1 min., 5 cycles of 94°C for 30 sec. and 72°C for 4 min.; 5 cycles of 94°C for 30 sec. and 70°C for 4 min.; 25 cycles of 94°C for 20 sec and 68°C for 4 min. An approximately 1.4 kb amplified product was identified which hybridized

with a ^{32}P-labeled probe derived from the TM 2-4 region (3F/4R probe) of the MTL-R. This product was subcloned into PCR-Script vector (Invitrogen) and sequenced.

As diagrammed in Figures 6A-C, DNA sequence analysis revealed two distinct sequences corresponding to alternative use of two splice donor sequences and a common splice acceptor sequence. These results were confirmed by transfecting the MTL-1 genomic construct containing the complete ORF interrupted by a single intron of approximately 0.7 kb into HEK-293/aeq17 cells. mRNA was the isolated (Poly $(A)^+$ Pure Kit, Ambion) and shown by Northern blot analysis using the 3F/4R probe to give two hybridizing bands: 2.4 kb containing the unspliced intron and approximately 1.4 kb encoding spliced forms. RT-PCR was then performed (Superscript 2 One-Step Kit, Life Technologies) on MTL-1 mRNA from transfected HEK-293/aeq17 cells using the forward primer 5' RACE1 and reverse primer 3' RACE2 (TM5 region): 5'-CTG CCC TTT CTG TGC CTC AGC ATC CTC TAC-3' (SEQ.ID.NO.:10)

An approximately 500 bp product was cloned (TA vector pCR2.2, Invitrogen), sequenced and shown to be a mixture of both splice forms. Assembly of the complete ORF for MTL-1A without intronic sequence was performed by ligation of an exon 1 fragment (Not I digestion of a MTL-1 plasmid containing the intron in pCDNA-3) to pCDNA-3.1 containing a Not 1/EcoR1 exon 2 fragment.

To document protein expression, an MTL-1A plasmid encoding a amino-terminal FLAG epitope was constructed by ligation of a Pme 1 fragment from the MTL-1A/pcDNA-1.1 vector into the EcoRV site of pFLAG/CMV-2 vector (Kodak Imaging Systems). Following transfection of this plasmid into HEK-293/aeq17 cells, a protein of the expected size (approximately 48 kDa) was detected in crude cell membranes by immunoblot analysis.

EXAMPLE 2

Identification of Ligand Specific to Motilin Receptor

To identify a ligand for this orphan GPCR and to determine whether the full length, 7 TM domain GPR38-A is a functional GPCR, a

high-throughput assay was developed which measures Ca^{2+} release with the bioluminescent Ca^{2+} sensitive aequorin reporter protein (capable of measuring ligand-induced IP_3-coupled mobilization of intracellular calcium and concomitant calcium-induced aequorin bioluminescence). Expression of GPR38-A in cell membranes was confirmed using epitope-tagged protein which revealed a single protein species with a molecular weight of approximately 45,000 daltons.

A broad set of peptide and non-peptide molecules was tested at a single concentration in transiently transfected HEK-293/aeq17 cells (100 nM peptide, 10 μM non-peptide). Significant bioluminescent responses (> 4-fold over background) were recorded for the peptide motilin and the non-peptide macrolide erythromycin, which was reported to be a competitive agonist at motilin receptors. Full dose-response curves confirmed this observation. The half-maximal effective concentration (EC_{50}) for human/porcine motilin was 2.1 +/- 0.5 nM motilin whereas erythromycin was considerably less potent (2000 +/- 210 nM; as expected from studies performed on native motilin receptors).

The signal tranduction pathway for the cloned GPR38-A motilin receptor (MTL-R1A) is through activation of phospholipase C, which has been reported for native motilin receptors. Direct radioligand binding studies with [^{125}I] human motilin on cell membranes prepared from transfected cells show that MTL-R1A confers high affinity and specific binding (K_d= 0.1 nM; B_{max}= 240 fmol/mg protein) which are strongly G protein coupled (> 80% inhibition of binding with 100 nM GTPγS) .

EXAMPLE 3

Functional Activation of the MTL-1A Receptor

The aequorin bioluminescence assay is a reliable test for identifying G protein-coupled receptors which couple through the Gα protein subunit family consisting of G_q and G_{11} which leads to the activation of phospholipase C, mobilization of intracellular calcium and activation of protein kinase C. Measurement of MTL-1A expression in the aequorin-expressing stable reporter cell line 293-AEQ17 (Button,

D. et. al.,1993 *Cell Calcium 14*: p. 663-671.) was performed using a Luminoskan RT luminometer (Labsystems Inc., Gaithersburg, MD).

293-AEQ17 cells (8 x 105 cells plated 18 hrs. before transfection in a T75 flask) were transfected with 22 µg of human MTL-R1A plasmid DNA: 264 µg lipofectamine. Following approximately 40 hours of expression the apo-aequorin in the cells was charged for 4 hours with coelenterazine (10 µM) under reducing conditions (300 µM reduced glutathione) in ECB buffer (140 mM NaCl, 20 mM KCl, 20 mM HEPES-NaOH [pH=7.4], 5 mM glucose, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 0.1 mg/ml bovine serum albumin). The cells were harvested, washed once in ECB medium and resuspended to 500,000 cells/ml. 100 µl of cell suspension (corresponding to $5x10^4$ cells) was then injected into the test plate, and the integrated light emission was recorded over 30 seconds, in 0.5 second units. 20 µL of lysis buffer (0.1% final Triton X-100 concentration) was then injected and the integrated light emission recorded over 10 seconds, in 0.5 second units. The "fractional response" values for each well were calculated by taking the ratio of the integrated response to the initial challenge to the total integrated luminescence including the Triton X-100 lysis response.

EXAMPLE 4

Binding of [^{125}I] Human Motilin to Crude Membranes from HEK-293 Cells transfected with the MTL-R1A cDNA.

The binding of [^{125}I] human motilin to crude membranes prepared from HEK-293/aeq17 cell transfectants was performed as follows. Crude cell membranes were prepared on ice, 48 hrs. post-transfection. Each T-75 flask was washed twice with 10 ml of PBS, once with 1 ml homogenization buffer (50 mM Tris-HCl [pH 7.4], 10 mM $MgCl_2$. 10 ml of homogenization buffer was added to each flask, cells were removed by scraping and then homogenized using a Polytron device (Brinkmann, Syosset, NY; 3 bursts of 10 sec. at setting 4). The homogenate was centrifuged for 20 min. at 11,000 x g at 0°C and the resulting crude membrane pellet (chiefly containing cell membranes and nuclei) was resuspended in homogenization buffer supplemented with 1.5 % BSA (0.5 ml T75 flask) and kept on ice.

Binding reactions were performed at 20°C for 1 hr. in a total volume of 0.5 ml containing: 0.1 ml of membrane suspension (approximately 1 μg protein), 10 μl of ^{125}I-human motilin, 10 μl of competing drug and 380-390 μl of homogenization buffer. Bound radioligand was separated by rapid vacuum filtration (Brandel 48-well cell harvester) through GF/C filters pretreated for 1 hr. with 0.5% polyethylenimine. After application of the membrane suspension to the filter, the filters were washed 3 times with 3 ml each of ice-cold 50 mM Tris-HCl [pH 7.4], 10 mM $MgCl_2$, and the bound radioactivity on the filters was quantitated by gamma counting. Specific binding (> 90% of total) is defined as the difference between total binding and non-specific binding conducted in the presence of 100 nM unlabeled human motilin. Competition binding data were analyzed by a nonlinear curve-fitting program (Prism V, version 2.0; GraphPad Software, San Diego, CA). Results shown are the means (+/- SEM) of triplicate determinations. Human motilin was radiolabeled with ^{125}I at ^{7}Tyr to a specific activity of approximately 2000 Ci/mmol (Woods Assay, Portland, OR).

Structure-function analysis suggest that the motilin peptide minimally contains an N-terminal region (amino acids 1-7) essential for activity, linked to a C-terminal alpha helical domain which stabilizes the N-terminal active site region activity. The rank order of potency of several motilin peptide analogs in the MTL1-A functional and binding assays correlates with their reported potency measured by *in vitro* contractility assays (Table 1) performed on native motilin receptors in intestinal tissue. These results are summarized in Table 1 below.

Ligand	Cloned MTL-1A Receptor (human)	
	Aequorin Assay (EC_{50} nM)	[^{125}I] hmotilin binding (IC_{50},nM)
human motilin (MTL)	2.1	0.5
erythromycin	2000	427
roxithromycin	1950	613
metoclopramide	>10,000	>10,000
cisapride	>10.000	>10,000

canine motilin	4.4	0.2
Leu13 MTL	3.9	0.2
1-11 MTL	138	127
1-12 MTL	72	14
1-13 MTL	3.8	0.9
1-19 MTL	4.1	0.3
10-22 MTL	>10,000	1100

The unrelated prokinetic agents metoclopromide and cisapride which have affinity for dopamine and/or 5-HT receptors were inactive, even at high (10 μM) doses.

EXAMPLE 5
Southern Blot Analysis

A genomic Southern blot (EcoRI and BamH1-digested DNA, 10 μg/lane) was hybridized with the ORF of MTL-1A. Post-hybridizational washing stringencies were at 55°C 4 X SSPE after which the filters were dried and exposed to X-ray film for 5 days at -70°C. Lambda Hind III DNA markers were (in kb), 23.1, 9.4, 6.6, 4.4, 2.3, 2.1. Southern blot analysis conducted in a variety of mammalian and non-mammalian species revealed a simple hybridization pattern consistent with a single, conserved gene encoding MTL-1A.

EXAMPLE 6
Puffer Fish Clone 75E7

Screening of a puffer fish genomic library identified a single clone (75E7) containing an open reading frame of 363 amino acids with approximately 54% protein sequence identity to the human MTL-R1A In addition, 75E7 has a similar intron-exon structure to the human MTL-R1A. 75E7 may be the ortholog of the human MTL-R1A.

EXAMPLE 7
Expression of the MTL-R1A Gene

Transcripts of MTL-1A were detected by RNase Protection Assay (RPA). Synthesis of high-specific activity radiolabeled antisense probes and the RPA was conducted using a kit (MAXIscript and HybSpeed RPA kits; Ambion, Austin, TX) essentially as described by the manufacturer. The anti-sense cRNA MTL-1A probe was synthesized from a cDNA template encompassing nt 1234 to 1516 of the human MTL-1A inserted behind the T7 promoter in pLitmus 28 (New England Biolabs, Beverly, MA). Digestion of the construct with Stu I generated a cRNA transcript approximately 340 nt in size with approximately 60 nt of vector sequence. Input poly A^+ mRNA (Clontech, Palo Alto, CA) was 5 g for the MTL-1A probe and 0.1 µg for a control human actin probe. Precipitated fragments were subjected to slab-gel electrophoresis (42 cm x 32 cm x 0.4 mm) in 5 % acrylamide/Tris-borate-EDTA buffer containing 8 M urea. The gels were fixed, dried and autoradiographed on film (X-Omat; Kodak, Rochester, NY) for 1-3 days (MTL-1A) or 2 hrs. (actin).

The distribution profile of MTL-1A mRNA was examined in a panel of GI and non-GI human tissues. MTL-1A mRNA could be detected in whole stomach (most prominently), thyroid, and bone marrow but was absent from several brain regions and other non-CNS tissues.

WHAT IS CLAIMED:

1. A motilin receptor, substantially free from receptor-associated proteins.

2. A motilin receptor according to Claim 1 which is human.

3. A motilin receptor according to Claim 2 which is MTL-R1A having the amino acid sequence SEQ.ID.NO.:3.

4. A motilin receptor according to Claim 3 having the nucleic acid sequence SEQ.ID.NO.:2.

5. A motilin receptor according to Claim 2 which is MTL-R1B having the amino acid sequence SEQ.ID.NO.:5.

6. A motilin receptor according to Claim 5 having the nucleic acid sequence SEQ.ID.NO.:4.

7. A motilin receptor according to Claim 6 which is 75E7 having the amino acid sequence SEQ.ID.NO.:7.

8. A method for determining whether a ligand is capable of binding to a motilin receptor comprising:

(a) transfecting test cells with an expression vector encoding motilin receptor;

(b) exposing the test cells to the ligand;

(c) measuring the amount of binding of the ligand to the motilin receptor;

(d) comparing the amount of binding of the ligand to the motilin receptor in the test cells with the amount of binding of the ligand to control cells that have not been transfected with the motilin receptor

where if the amount of binding of the ligand to the test cells is greater than the amount of binding of the ligand to the control cells, then the substance is capable of binding to motilin receptor.

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C07H 21/04, C07K 14/705, C12N 15/09, 15/63, C12Q 1/68

A1

(11) International Publication Number: WO 99/64436

(43) International Publication Date: 16 December 1999 (16.12.99)

(21) International Application Number: PCT/US99/12773

(22) International Filing Date: 8 June 1999 (08.06.99)

(30) Priority Data:
60/089,098 12 June 1998 (12.06.98) US

(71) Applicant (*for all designated States except US*): MERCK & CO., INC. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US).

(72) Inventors; and
(75) Inventors/Applicants (*for US only*): FEIGHNER, Scott, D. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). PATCHETT, Arthur, A. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). TAN, Carina [MY/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). MC-KEE, Karen [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). MACNEIL, Douglas [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). HOWARD, Andrew, D. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). PONG, Sheng-Shung [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). SMITH, Roy, G. [GB/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US).

(74) Common Representative: MERCK & CO., INC.; 126 East Lincoln Avenue, Rahway, NJ 07065 (US).

(81) Designated States: CA, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) **Title:** CLONING AND IDENTIFICATION OF THE MOTILIN RECEPTOR

(57) **Abstract**

The motilin receptor has been isolated and cloned, and nucleic acid sequences are given. Two splice variants have been identified. Also, assays for motilin receptor ligands are given. The identification of the cloned motilin receptor may be used to screen and identify compounds which bind to the receptor for use in a variety of gastric conditions, including gastric motility disorders.

TTGAAATTATCTGGTCACTGCCGGGCGCGGTGGCTCACGCCTGTAATCCCAGCACTTTGGGAGGTCGA
GGCGGGTGGACCACCTGGGGTCAGGAGTTCGAGACCAGGCTGGCCAACATGGCGAAACCCTGACTACA
CAAAAAACACAAAATTTAGCCGGGGCTTGGGCGCTCCTGTGCTCCCAGCTACTCAGGAGGCTGAGGTG
GGAGGACTGCTTGAGCCTGGGAGGTCGAGGCTGCAGTGAGCTGTGATCGCGCCACTTAAACTCCAGCC
TGGACGACAGTGAGACCCTGTCTCAAGAAGAAAAAAAGAAAGAAAGAAAGAAAAAAAGAAAAAAAAGA
AATTATTTGGTCAATTATATGGTCAGCTCCCTCCACCACTCGCGAATTTACAGAAGAGGAGAACTGGG
CTGGGCGAGACCAGGACTAGCCCAAGATTACACAAGTTACTCGGTTGTAGAGCCAGGATTAGACAGGA
GAGGCTCTAGATTCTGGTCTAGACTCCCCTCCTATTATTTAGCATTATGGCTTCCTGAGGATTACCAT
GAGCCCTCCTCCACCGTCAAGCGGCAGCTACCAGCCACCAGACCAGATCCCTTCGAAGGTGCCCGGAG
TACCAGACTGACAAAAGCGCCCGTACAGTGCTCAGTCCTGTAACCAAAGCTGTCTAGGGTGCAGACAT
CGCTCACCGGACCGGGTAGGGCTCGTGCGCTAAGGGCGCCGGGTATTCCAGTTAGTGGAGAGGGAAGC
GCCCTGGAACTGCATGGGCCCGGGAGAGGGCGCGGGAGCGGAGCATGGCCGGGCCGGGGCGGCCGCG
GCCGTGGGCGGAGACTGCGCGCAGCTAGCTCGGGAGCGCCTCGGAGCC QCCCCGCAGAGCCGCTTCT
CGCGCCCCGCAGCGCAGCGCAGCGCTCCGCCGTCTGACCTGCCGCGCCCGCAGCGTGCGGGCTGGGAA
AGGAGGCGCTCACCGAGAGGGACCACGCGCCAGGCTCCCAGCCCGACCCGGGACGCGGCGGCCGCGCG
GAGCACCCATGGGCAGCCCCTGGAACGGCAGCGACGGCCCCGAGGGGGCGCGGGAGCCGCCGTGGCCC
GCGCTGCCGCCTTGCGACGAGCGCCGCTGCTCGCCCTTTCCCCTGGGGGCGCTGGTGCCGGTGACCGC
TGTGTGCCTGTGCCTGTTCGTCGTCGGGGTGAGCGGCAACGTGGTGACCGTGATGCTGATCGGGCGCT
ACCGGGACATGCGGACCACCACCAACTTGTACCTGGGCAGCATGGCCGTGTCCGACCTACTCATCCTG
CTCGGGCTGCCGTTCGACCTGTACCGCCTCTGGCGCTCGCGGCCCTGGGTGTTCGGGCCGCTGCTCTG
CCGCCTGTCCCTCTACGTGGGCGAGGGCTGCACCTACGCCACGCTGCTGCACATGACCGCGCTCAGCG
TCGAGCGCTACCTGGCCATCTGCCGCCCGCTCCGCGCCCGCGTCTTGGTCACCCGGCGCCGCGTCCGC
GCGCTCATCGCTGTGCTCTGGGCCGTGGCGCTGCTCTCTGCCGGTCCCTTCTTGTTCCTGGTGGGCGT
CGAGCAGGACCCCGGCATCTCCGTAGTCCCGGGCCTCAATGGCACCGCGCGGATCGCCTCCTCGCCTC
TCGCCTCGTCGCCGCCTCTCTGGCTCTCGCGGGCGCCACCGCCGTCCCCGCCGTCGGGGCCCGAGACC
GCGGAGGCCGCGGCGCTGTTCAGCCGCGAATGCCGGCCGAGCCCCGCGCAGCTGGGCGCGCTGCGTGT
CATGCTGTGGGTCACCACCGCCTACTTCTTCCTGCCCTTTCTGTGCCTCAGCATCCTCTACGGGCTCA
TCGGGCGGGAGCTGTGGAGCAGCCGGCGGCCGCTGCGAGGCCCGGCCGCCTCGGGGCGGGAGAGAGGC
CACCGGCAGACCGTCCGCGTCCTGCgtaagtggagccgccgtggttccaaagacgcctgcctgcagtc
cgccccgccggggaccgcgcaaacgctccctccccttcccctgctcgcccagctctgggcgccgcttc
cagctcccttcctatttcgattccagcctccacccgccggtcattcccatcccccgagaaaaccatgt
cctgtcccccaggagctctgggggaccccagggcgctttgagggtgggatccccggatccgattcagt
aaccagcagtgcttttccagagcctctgagaccagaaaggagagttggtaattcttaatccaaccacc
tgttagatgccacaaatgaggagtcctcacagtgctcttgagaagacgagggagatttcattaagcta
aaatttttatttaatgttaagtgatgctgaaggctaaagtaaaccttgctcgtatcaaaaagtaaag
attgtgcagacctgttgtagaattcttttcaacagagaacagaaaacttgtctccgaagtgggtttgt
ggaaggaagcctgccaaggcggcttgttcagagaaattgctccttctggtttatgtccagccttgata
acacatatgggagcctactatgcagttttaaagcaagtatccatgcagcctgcagcctggtcattttt
tctggggtgaggatctgcctaggtagaagttttctctaatttattttgctgttacttgttattgcaga
tggttccttgtcggggtgggggtttatttgcttcccaatgcttttgttaatcccggtgctgtgtctt
atgttgcagTGGTGGTGGTTCTGGCATTTATAATTTGCTGGTTGCCCTTCCACGTTGGCAGAATCATT
TACATAAACACGGAAGATTCGCGGATGATGTACTTCTCTCAGTACTTTAACATCGTCGCTCTGCAACT
TTTCTATCTGAGCGCATCTATCAACCCAATCCTCTACAACCTCATTTCAAAGAAGTACAGAGCGGCGG
CCTTTAAACTGCTGCTCGCAAGGAAGTCCAGGCCGAGAGGCTTCCACAGAAGCAGGACACTGCGGGG
GAAGTTGCAGGGGACACTGGAGGAGACACGGTGGGCTACACCGAGACAAGCGCTAACGTGAAGACGAT
GGGATAA

FIG. 1

ATGGGCAGCCCCTGGAACGGCAGCGACGGCCCCGAGGGGGCGCGGGAGCCGCCGTGGCCCGCGCTG
CCGCCTTGCGACGAGCGCCGCTGCTCGCCCTTTCCCCTGGGGGCGCTGGTGCCGGTGACCGCTGTG
TGCCTGTGCCTGTTCGTCGTCGGGGTGAGCGGCAACGTGGTGACCGTGATGCTGATCGGGCGCTAC
CGGGACATGCGGACCACCACCAACTTGTACCTGGGCAGCATGGCCGTGTCCGACCTACTCATCCTG
CTCGGGCTGCCGTTCGACCTGTACCGCCTCTGGCGCTCGCGGCCCTGGGTGTTCGGGCCGCTGCTC
TGCCGCCTGTCCCTCTACGTGGGCGAGGGCTGCACCTACGCCACGCTGCTGCACATGACCGCGCTC
AGCGTCGAGCGCTACCTGGCCATCTGCCGCCCGCTCCGCGCCCGCGTCTTGGTCACCCGGCGCCGC
GTCCGCGCGCTCATCGCTGTGCTCTGGGCCGTGGCGCTGCTCTCTGCCGGTCCCTTCTTGTTCCTG
GTGGGCGTCGAGCAGGACCCCGGCATCTCCGTAGTCCCGGGCCTCAATGGCACCGCGCGGATCGCC
TCCTCGCCTCTCGCCTCGTCGCCGCCTCTCTGGCTCTCGCGGGCGCCACCGCCGTCCCCGCCGTCG
GGGCCCGAGACCGCGGAGGCCGCGGCGCTGTTCAGCCGCGAATGCCGGCCGAGCCCCGCGCAGCTG
GGCGCGCTGCGTGTCATGCTGTGGGTCACCACCGCCTACTTCTTCCTGCCCTTTCTGTGCCTCAGC
ATCCTCTACGGGCTCATCGGGCGGGAGCTGTGGAGCAGCCGGCGGCCGCTGCGAGGCCCGGCCGCC
TCGGGGCGGGAGAGAGGCCACCGGCAGACCGTCCGCGTCCTGCTGGTGGTGGTTCTGGCATTTATA
ATTTGCTGGTTGCCCTTCCACGTTGGCAGAATCATTTACATAAACACGGAAGATTCGCGGATGATG
TACTTCTCTCAGTACTTTAACATCGTCGCTCTGCAACTTTTCTATCTGAGCGCATCTATCAACCCA
ATCCTCTACAACCTCATTTCAAAGAAGTACAGAGCGGCGGCCTTTAAACTGCTGCTCGCAAGGAAG
TCCAGGCCGAGAGGCTTCCACAGAAGCAGGGACACTGCGGGGGAAGTTGCAGGGGACACTGGAGGA
GACACGGTGGGCTACACCGAGACAAGCGCTAACGTGAAGACGATGGGATAA

FIG.2

MGSPWNGSDGPEGAREPPWPALPPCDERRCSPFPLGALVPVTAVCLCLFVVGVSGNVVTVMLIGRY
RDMRTTTNLYLGSMAVSDLLILLGLPFDLYRLWRSRPWVFGPLLCRLSLYVGEGCTYATLLHMTAL
SVERYLAICRPLRARVLVTRRRVRALIAVLWAVALLSAGPFLFLVGVEQDPGISVVPGLNGTARIA
SSPLASSPPLWLSRAPPPSPPSGPETAEAAALFSRECRPSPAQLGALRVMLWVTTAYFFLPFLCLS
ILYGLIGRELWSSRRPLRGPAASGRERGHRQTVRVLLVVVLAFIICWLPFHVGRIIYINTEDSRMM
YFSQYFNIVALQLFYLSASINPILYNLISKXYRAAAFKLLLARKSRPRGFHRSRDTAGEVAGDTGG
DTVGYTETSANVKTMG

FIG.3

ATGGGCAGCCCCTGGAACGGCAGCGACGGCCCCGAGGGGGCGCGGAGCCGCCGTGGCCCGCGCTG
CCGCCTTGCGACGAGCGCCGCTGCTCGCCCTTTCCCCTGGG&GCGCTGGTGCCGGTGACCGCTGTG
TGCCTGTGCCTGTTCGTCGTCGGGGTGAGCGGCAACGTGGTGACCGTGATGCTGATCGGGCGCTAC
CGGGACATGCGGACCACCACCAACTTGTACCTGGGCAGCATGGCCGTGTCCGACCTACTCATCCTG
CTCGGGCTGCCGTTCGACCTGTACCGCCTCTGGCGCTCGCGGCCCTGGGTGTTCGGGCCGCTGCTC
TGCCGCCTGTCCCTCTACGTGGGCGAGGGCTGCACCTACGCCACGCTGCTGCACATGACCGCGCTC
AGCGTCGAGCGCTACCTGGCCATCTGCCGCCCGCTCCGCGCCCGCGTCTTGGTCACCCGGCGCCGC
GTCCGCGCGCTCATCGCTGTGCTCTGGGCCGTGGCGCTGCTCTCTGCCGGTCCCTTCTTGTTCCTG
GTGGGCGTCGAGCAGGACCCCGGCATCTCCGTAGTCCCGGGCCTCAATGGCACCGCGCGGATCGCC
TCCTCGCCTCTCGCCTCGTCGCCGCCTCTCTGGCTCTCGCGGGCGCCACCGCCGTCCCCGCCGTCG
GGGCCCGAGACCGCGGAGGCCGCGGCGCTGTTCAGCCGCGAATGCCGGCCGAGCCCCGCGCAGCTG
GGCGCGCTGCGTGTCATGCTGTGGGTCACCACCGCCTACTTCTTCCTGCCCTTTCTGTGCCTCAGC
ATCCTCTACGGGCTCATCGGGCGGGAGCTGTGGAGCAGCCGGCGGCCGCTGCGAGGCCCGGCCGCC
TCGGGGCGGGAGAGAGGCCACCGGCAGACCGTCCGCGTCCTGCGTAAGTGGAGCCGCCGTGGTTCC
AAAGACGCCTGCCTGCAGTCCGCCCCGCCGGGGACCGCGCAAACGCTGGGTCCCCTTCCCCTGCTC
GCCCAGCTCTGGGCGCCGCTTCCAGCTCCCTTTCCTATTTCGATTCCAGCCTCCACCCGCCGTGGT
GGTGGTTCTGGCATTTATAATTTGCTGGTTGCCCTTCCACGTTGGCAGAATCATTTACATAAACAC
GGAAGATTCGCGGATGATGTACTTCTCTCAGTACTTTAACATCGTCGCTCTGCAACTTTTCTATCT
GAGCGCATCTATCAACCCAATCCTCTACAACCTCATTTCAAAGAAGTACAGAGCGGCGGCCTTTAA
ACTGCTGCTCGCAAGGAAGTCCAGGCCGAGAGGCTTCCACAGAAGCAGGACACTGCGGGGGAAGT
TGCAGGGGACACTGGAGGAGACACGGTGGGCTACACCGAGACAAGCGCTAACGTGAAGACGATGGG
ATAA

FIG.4

MGSPWNGSDGPEGAREPPWPALPPCDERRCSPFPLGALVPVTAVCLCLFVVGVSGNVVIVMLIGRY
RDMRTTTNLYLGSMAVSDLLILLGLPFDLYRLWRSRPWVFGPLLCRLSLYVGEGCTYATLLHMTAL
SVERYLAICRPLRARVLVTRRRVRALIAVLWAVALLSAGPFLFLVGVEQDPGISVVPGLNGTARIA
SSPLASSPPLWLSRAPPPSPPSGPETAEAAALFSRECRPSPAQLGALRVMLWVTTAYFFLPFLCLS
ILYGLIGRELWSSRRPLRGPAASGRERGHRQTVRVLRKWSRRGSKDACLQSAPPGTAQTLGPLPLL
AQLWAPLPAPFPISIPASTRRGGGSGIYNLLVALPRWQNHLHKHGRFADDVLLSVL

FIG.5

```
  ATG GGC AGC CCC TGG AAC GGC AGC GAC GGC CCC GAG GGG GCG CGG GAG CCG CCG TGG CCC GCG CTG CCG CCT TGC
1 M   G   S   P   W   N   G   S   D   G   P   E   G   A   R   E   P   P   W   P   A   L   P   P   C
                                              TM1
  GAC GAG CGC CGC TGC TCG CCC TTT CCC CTG GGG GCG CTG GTG CCG GTG ACC GCT GTG TGC CTG TGC CTG TTC GTC
  D   E   R   R   C   S   P   F   P   L   G   A   L   V   P   V   T   A   V   C   L   C   L   F   V

  GTC GGG GTG AGC GGC AAC GTG GTG ACC GTG ATG CTG ATC GGG CGC TAC CGG GAC ATG CGG ACC ACC ACC AAC TTG
  V   G   V   S   G   N   V   V   T   V   M   L   I   G   R   Y   R   D   M   R   T   T   T   N   L
              TM2
  TAC CTG GGC AGC ATG GCC GTG TCC GAC CTA CTC ATC CTG CTC GGG CTG GAC TTC GAC CTG TAC CGC CTC TGG CGC
  Y   L   G   S   M   A   V   S   D   L   L   I   L   L   G   L   P   F   D   L   Y   R   L   W   R
                                              TM3
  TCG CGG CCC TGG GTG TTC GGG CCG CTG CTC TGC CGC CTG TCC CTC TAC GTG GGC GAG GGC TGC ACC TAC GCC ACG
  S   R   P   W   V   F   G   P   L   L   C   R   L   S   L   Y   V   G   E   G   C   T   Y   A   T

  CTG CTG CAC ATG ACC GCG CTC AGC GTC GAG CGC TAC CTG GCC ATC TGC CGC CCG CTC CGC GCC CGC GTC TTG GTC
  L   L   H   M   T   A   L   S   V   E   R   Y   L   A   I   C   R   P   L   R   A   R   V   L   V
                                  TM4
  ACC CGG CGC CGC GTC CGC GCG CTC ATC GCT GTG CTC TGG GCC GTG GCG CTG CTC TCT GCC GGT CCC TTC TTG TTC
  T   R   R   R   V   R   A   L   I   A   V   L   W   A   V   A   L   L   S   A   G   P   F   L   F

  CTG GTG GGC GTC GAG CAG GAC CCC GGC ATC TCC GTA GTC CCG GGC CTC AAT GGC ACC GCG CGG ATC GCC TCC TCG
  L   V   G   V   E   Q   D   P   G   I   S   V   V   P   G   L   N   G   T   A   R   I   A   S   S

  CCT CTC GCC TCG TCG CCG CCT CTC TGG CTC TCG CGG GCG CCA CCG CCG TCC CCG CCG TCG GGG CCC GAG ACC GCG
  P   L   A   S   S   P   P   L   W   L   S   R   A   P   P   P   S   P   P   S   G   P   E   T   A
                                                                                      TM5
  GAG GCC GCG GCG CTG TTC AGC CGC GAA TGC CGG CCG AGC CCC GCG CAG CTG GGC GCG CTG CGT GTC ATG CTG TGG
  E   A   A   A   L   F   S   R   E   C   R   P   S   P   A   Q   L   G   A   L   R   V   M   L   W

  GTC ACC ACC GCC TAC TTC TTC CTG CCC TTT CTG TGC CTC AGC ATC CTC TAC GGG CTC ATC GGG CGG GAG CTG TGG
  V   T   T   A   Y   F   F   L   P   F   L   C   L   S   I   L   Y   G   L   I   G   R   E   L   W

  AGC AGC CGG CGG CCG CTG CGA GGC CCG GCC GCC TCG GGG CGG GAG AGA GGC CAC CGG CAG ACC GTC CGC GTC CTG
  S   S   R   R   P   L   R   G   P   A   A   S   G   R   E   R   G   H   R   Q   T   V   R   V   L
```

FIG.6A

(Donor A)
CgtAAGTGGAGCCGCCGTGGTTCCAAAGACGCCTGCCTGCAGTCCGCCCCGCCGGGGACCGCGCAAACGCTGGGTCCCCT
TCCCCTGCTCGCCCAGCTCTGGGCGCCGCTTCCAGCTCCCTTTCCTATTTCGATTCCAGCCTCCACCCGCCGgt...+569 bp
(Donor B)

FM-1A: 7TM, 403 amino acids

```
          TM6
ag/CTG GTG GTG GTT CTG GCA TTT ATA ATT TGC TGG TTG CCC TTC CAC GTT GGC AGA ATC
    L   V   V   V   L   A   F   I   I   C   W   L   P   F   H   V   O   R   I

                                TM7
ATT TAC ATA AAC ACG GAA GAT TCG CGG ATG ATG TAC TTC TCT CAG TAC TTT AAC ATC GTC GCT CTG CAA CTT TTC
 I   Y   I   N   T   E   D   S   R   M   M   Y   F   S   Q   Y   F   N   I   V   A   L   Q   L   F

TAT CTG AGC GCA TCT ATC AAC CCA ATC CTC TAC AAC CTC ATT TCA AAG AAG TAC AGA GCG GCG GCC TTT AAA CTG
 Y   L   S   A   S   I   N   P   I   L   Y   N   L   I   S   K   K   Y   R   A   A   A   F   K   L

CTG CTC GCA AGG AAG TCC AGG CCG AGA GGC TTC CAC AGA AGC AGG GAC ACT GCG GGG GAA GTT GCA GGG GAC ACT
 L   L   A   R   K   S   R   P   R   G   F   H   R   S   R   D   T   A   G   E   V   A   G   D   T

GGA GGA GAC ACG GTG GGC TAC ACC GAG ACA AGC GCT AAC GTG AAG ACG ATG GGA TAA
 G   G   D   T   V   G   Y   T   E   T   S   A   N   V   K   T   M   G   *          403
```

FIG.6B

FM-1B: 5TM, 387 amino acids

```
  CGT AAG TGG AGC CGC CGT GGT TCC AAA GAC GCC TGC CTG CAG TCC GCC CCG CCG GGG ACC GCG CAA ACG CTG
  R   K   W   S   R   R   G   S   K   D   A   C   L   Q   S   A   P   P   G   T   A   Q   T   L

GGT CCC CTT CCC CTG CTC GCC CAG CTC TGG GCG CCG CTT CCA GCT CCC TTT CCT ATT TCG ATT CCA GCC TCC ACC
G   P   L   P   L   L   A   Q   L   W   A   P   L   P   A   P   F   P   I   S   I   P   A   S   T

CGC CGT GGT GGT GGT TCT GGC ATT TAT AAT TTG CTG GTT GCC CTT CCA CGT TGG CAG AAT CAT TTA CAT AAA CAC
R   R   G   G   G   S   G   I   Y   N   L   L   V   A   L   P   R   W   Q   N   H   L   H   K   H

GGA AGA TTC GCG GAT GAT GTA CTT CTC TCA GTA CTT TAA
G   R   F   A   D   D   V   L   L   S   V   L   *                                             387
```

FIG.6C

ATGCCCTGGACCAGACCCCAGGTGGACCTCCATGCTGCTGCAGCAGAGACCATGGACCAGTACACC
ACGGACGACCACCACTACGAGGGCTCCCTCTTCCCCGCGTCCACCCTCATCCCCGTCACGGTCATC
TGCATCCTCATCTTCGTGGTCGGCGTGACCGGCAACACCATGACCATCCTCATCATCCAGTACTTC
AAGGACATGAAGACCACCACCAACCTGTACCTGTCCAGCATGGCCGTGTCCGACCTCGTCATCTTC
CTCTGCCTGCCCTTCGACCTGTACCGCCTGTGGAAGTACGTGCCGTGGCTGTTCGGCGAGGCCGTG
TGCCGCCTCTACCACTACATCTTCGAAGGCTGCACGTCGGCCACCATCCTCCACATCACGGCCCTG
AGCATCGAGCGCTACCTGGCCATCAGCTTCCCCCTCAGGAGCAAGGTGATGGTGACCAGGAGAAGG
GTCCAGTACATCATCCTGGCCCTGTGGTGCTTCGCCCTGGTGTCGGCCGCTCCCACGCTCTTCCTG
GTCGGGGTGGAGTACGACAACGAGACGCACCCCGACTACAACACGGGCCAGTGCAAGCACACGGGC
TACGCCATCAGCTCGGGGCAGCTGCACATCATGATCTGGGTGTCCACCACCTACTTCTTCTGCCCG
ATGCTGTGTCTCCTCTTCCTCTACGGCTCCATCGGGTGCAAGCTGTGGAAGAGCAAGAACGACCTG
CAGGGCCCGTGCGCCCTGGCCCGCGAGAGGTCGCACAGGCAAACGGTGAAGATCCTGGTGGTGGTG
GTGCTGGCCTTCATCATCTGCTGGCTGCCCTACCACATCGGCAGGAACCTGTTCGCCCAGGTGGAC
GACTACGACACGGCCATGCTCAGCCAGAATTTCAACATGGCCTCCATGGTGCTCTGCTACCTCAGC
GCCTCCATCAACCCCGTCGTCTACAACCTGATGTCGAGGAAGTACCGGGCCGCCGCCAAGCGCCTC
TTCCTGCTCCACCAGAGACCCAAGCCGGCCCACCGGGGGCAGGGGCAGTTTTGCATGATCGGCCAC
AGCCCCACCCTGGACGAGAGCCTGACGGGGGTGTGA

FIG.7

MPWTRPQVDLHAAAAETMDQYTTDDHHYEGSLFPASTLIPVTVICILIF W GVTGNT
MTILIIQYFKDMKTTTNLYLSSMAVSDLVIFLCLPFDLYRLWKYVPWLFGEAVCRLY
HYIFEGCTSATILHITALSIERYLAISFPLRSKVMVTRRRVQYIILALWCFALVSAA
PTLFLVGVEYDNETHPDYNTGQCKHTGYAISSGQLHIMIWVSTTYFFCPMLCLLFLY
GSIGCKLWKSKNDLQGPCALARERSHRQTVKILVVVVLAFIICWLPYHIGRNLFAQV
DDYDTAMLSQNFNMASMVLCYLSASINPVVYNLMSRKYRAAAKRLFLLHQRPKPAHR
GQGQFCMIGHSPTLDESLTGV

FIG.8

```
pu75E7 1 ..MPWTRPQVDLHAAAAETMDQYTTDDHHYEGSLFPASTLIPVTVICILI 48
           ||    |    | |              | ||   |:|||  :|: :
huMTLR 1 MGSPWNGS..DGPEGAREPPWPALPPCDERRCSPFPLGALVPVTAVCLCL 48

      49 FVVGVTGNTMTILIIQYFKDMKTTTNLYLSSMAVSDLVIFLCLPFDLYRL 98
         |||||.|| .|:::|  ::||:||||||| |||||||.| ||||||||||
      49 FVVGVSGNVVTVMLIGRYRDMRTTTNLYLGSMAVSDLLILLGLPFDLYRL 98

      99 WKYVPWLFGEAVCRLYHYIFEGCTSATILHITALSIERYLAISFPLRSKV 148
         |:  ||.||  .|||  |: |||| ||:||.||||:||||||  |||.:|
      99 WRSRPWVFGPLLCRLSLYVGEGCTYATLLHMTALSVERYLAICRPLRARV 148

     149 MVTRRRVQYIILALWCFALVSAAPTLFLVGVEYD................ 182
         :|||||. :|  ||  ||.|| | ||||||| |
     149 LVTRRRVRALIAVLWAVALLSAGPFLFLVGVEQDPGISVVPGLNGTARIA 198

     183 .............NETHPDYNTGQCKHTGYAISS..........GQLHIM 209
                          |   .|         |: |          | | :|
     199 SSPLASSPPLWLSRAPPPSPPSGPETAEAAALFSRECRPSPAQLGALRVM 248

     210 IWVSTTYFFCPMLCLLFLYGSIGCKLWKSKNDLQGPCALARERSHRQTVK 259
         :||.| ||| | |||  ||| || .|| |:  |.|| |   ||| |||||:
     249 LWVTTAYFFLPFLCLSILYGLIGRELWSSRRPLRGPAASGRERGHRQTVR 298

     260 ILVVVVLAFIICWLPYHIGRNLFAQVDDYDTAMLSQNFNMASMVLCYLSA 309
         :|.||||||||||||:|:||  ::  :|       || ||. .: | ||||
     299 VLLVVVLAFIICWLPFHVGRIIYINTEDSRMMYFSQYFNIVALQLFYLSA 348

     310 SINPVVYNIMSRRYRAAAKRLFLLHQ.RPKPAHRGQ...GQFCMIGHSPT 355
         ||||:.|||.|:||||||  :| |  . ||:  ||  .    |:       |
     349 SINPILYNLISKKYRAAAFKLLLARKSRPRGFHRSRDTAGEVAGDTGGDT 398

     356 LDESLTGV......                                     363
         .  . |
     399 VGYTETSANVKTMG                                     412
```

FIG.9

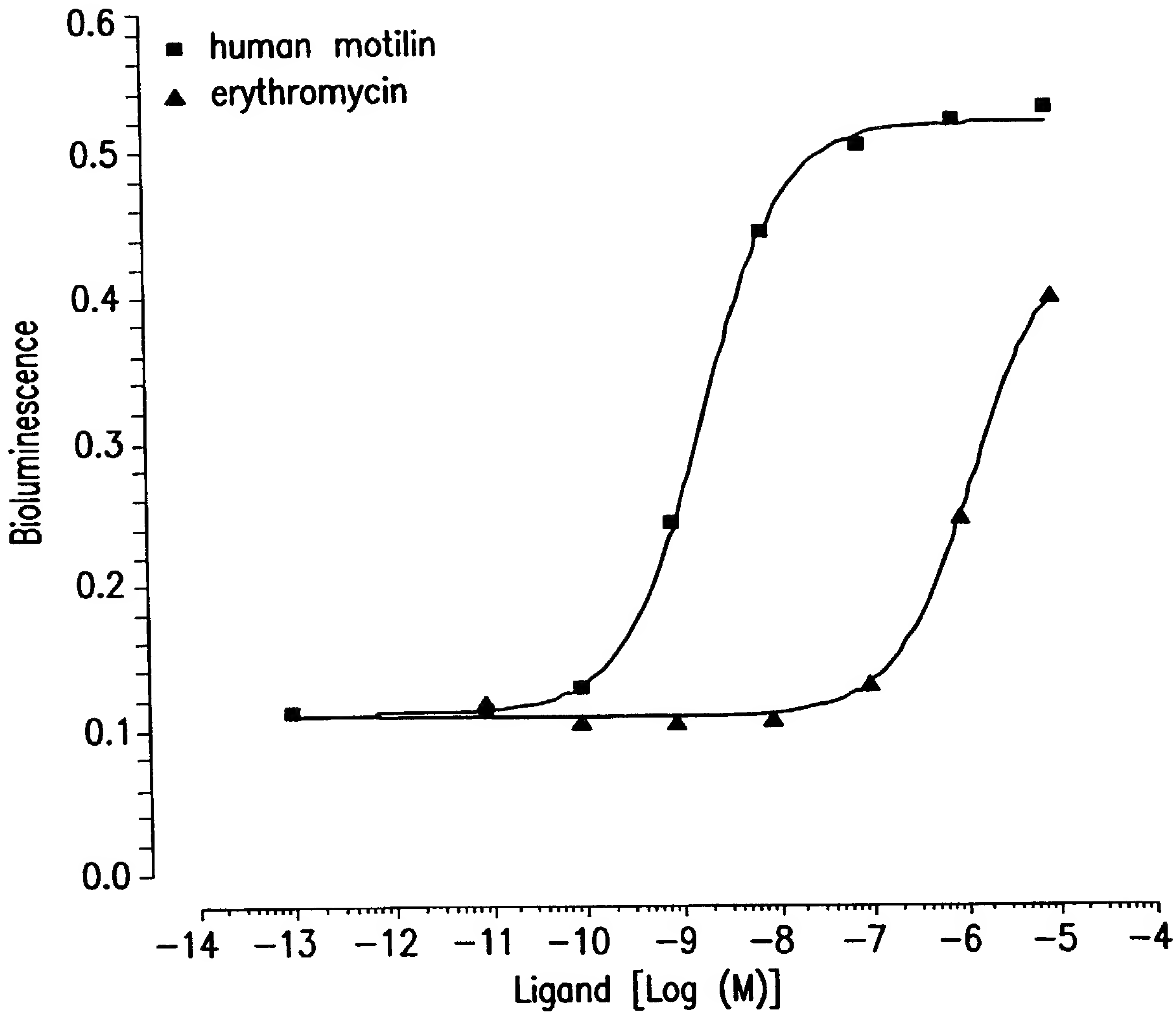
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Bioluminescence
human motilin
erythromycin
−14 −13 −12 −11 −10 −9 −8 −7 −6 −5 −4
Ligand [Log (M)]

FIG.10

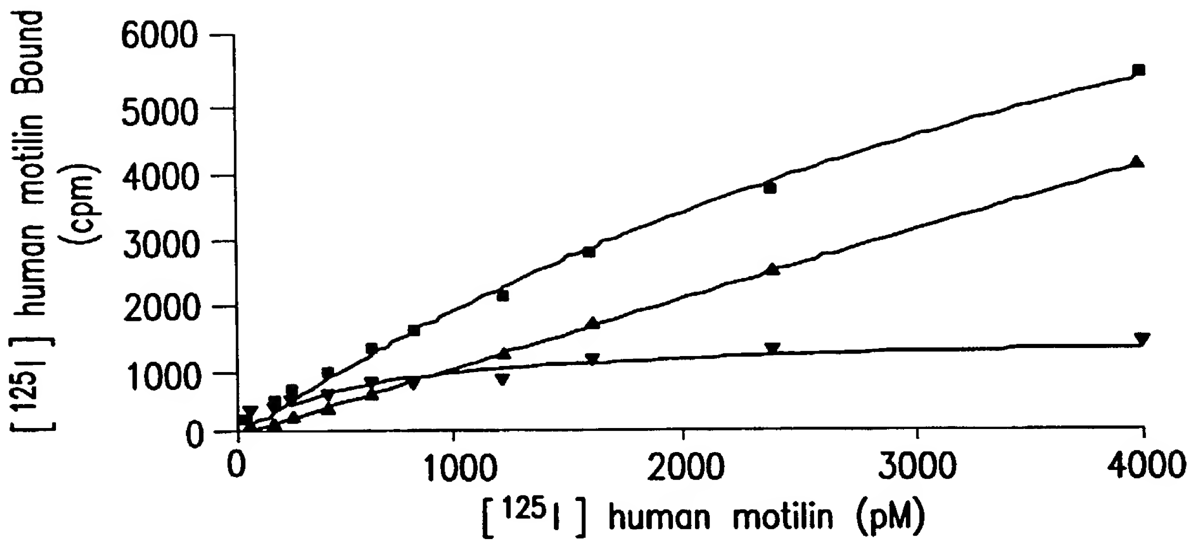
[125I] human motilin Bound (cpm)
6000
5000
4000
3000
2000
1000
0
0
1000
2000
3000
4000
[125I] human motilin (pM)

FIG. 11

Please type a plus sign (+) inside this box [+]

09719485 .052501

Approved for use through 9/30/2000 OMB 651-0032

SUBSTITUTE for PTO/SB/01 (12-97), DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION

#10

DECLARATION AND POWER OF ATTORNEY FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.63)

☐ Declaration Submitted with Initial Filing **OR** ☐ Declaration Submitted after Initial Filing (surcharge (37 CFR 1.16 (e)) required)

Attorney Docket Number	20251P
First Named Inventor	Feighner, Scott D. et al.
COMPLETE IF KNOWN	
Application Number	09/719,485
Filing Date	12/12/2000
Group Art Unit	
Examiner Name	

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

CLONING AND IDENTIFICATION OF THE MOTILIN RECEPTOR

(Title of the Invention)

the specification of which

☐ is attached hereto

OR

☒ was filed on (MM/DD/YYYY) 12/12/2000 as United States Application Number or PCT International Application Number 09/719,485 and was amended on (MM/DD/YYYY) ______ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose to the Patent and Trademark Office all information known to me to be material to patentability as defined in 37 CFR 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Attorney Docket Number	Priority Claimed? YES	NO
				☐	☐
				☐	☐
				☐	☐
				☐	☐

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto

I hereby claim the benefit under 35 U.S.C 119(e) of any United States provisional application(s) listed below.

Application Number(s)	Filing Date (MM/DD/YYYY)	Attorney Docket Number
60/089,098	06/12/1998	20251PV

Computer generated form " Declaration Pwr of Atty" (Application Filing Folder), Merck & Co., Inc. 04/05/2001

Please type a plus sign (+) inside this box → [+]

Approved for use through 9/30/2000 OMB 651-0032

SUBSTITUTE for PTO/SB/01 (12-97), DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION

DECLARATION AND POWER OF ATTORNEY for Utility or Design Patent Application

I hereby claim the benefit under 35 U.S.C 120 of any United States application(s), or 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of 35 U.S.C. 112, I acknowledge the duty to disclose information known to me to be material to patentability as defined in 37 CFR 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. Parent Application or PCT Parent Application Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number (*if applicable*)
PCT/US99/12733	06/08/1999	

☐ Additional U S or PCT international application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

As a named inventor, I hereby appoint, respectively and individually, as my attorneys or agents with full power of substitution and revocation, the following registered practitioner(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

☐ Customer Number [ ] → *Place Customer Number Bar Code Label here*
OR
☒ Registered practitioner(s) name/registration number listed below

Name	Registration Number	Name	Registration Number
Anna L. Cocuzzo	42,452		
Jack L. Tribble	32,633		

20

Direct all correspondence to: ☒ Customer Number or Bar Code Label 000210

Name	Anna L. Cocuzzo				
Address	Merck & Co., Inc. - Patent Department				
Address	P.O. Box 2000, RY60-30				
City	Rahway	**State**	NJ	**ZIP**	07065-0907
Country	USA	**Telephone**	(732)594-1273	**Fax**	(732)594-4720

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Name of Sole or First Inventor: ☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any])	Family Name or Surname
Scott D.	Feighner

1-00

Inventor's Signature	Scott D. Feighner			**Date**	23 MAY 01		
Residence: City	Holmdel	**State**	NJ NJ	**Country**	USA	**Citizenship**	US
Post Office Address	Merck & Co., Inc., P.O Box 2000						
City	Rahway	**State**	NJ	**ZIP**	07065-0907		

☐ Additional inventors are being named on the _____ supplemental Additional Inventors(s) sheet(s) PTO/SB/02A attached hereto.

Computer generated form " Declaration Pwr of Atty" (Application Filing Folder), Merck & Co., Inc. 05/23/2000

DECLARATION AND POWER OF ATTORNEY	ADDITIONAL INVENTOR(S) Supplemental Sheet

2-00

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor						
Given Name (first and middle [if any])				Family Name or Surname			
Arthur A.				Patchett			
Inventor's Signature	[signature]			**Date**	May 15, 2001		
Residence: City	Westfield	**State**	NJ NJ	**Country**	USA	**Citizenship**	US
Post Office Address	Merck & Co., Inc., P.O. Box 2000						
City	Rahway	**State**	NJ	**ZIP**	07065-0907		

3-00

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor						
Given Name (first and middle [if any])				Family Name or Surname			
Carina				Tan			
Inventor's Signature	[signature]			**Date**	May 21 2001		
Residence: City	Metuchen	**State**	NJ NJ	**Country**	USA	**Citizenship**	Malaysian
Post Office Address	Merck & Co., Inc., P.O. Box 2000						
City	Rahway	**State**	NJ	**ZIP**	07065-0907		

4-00

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor						
Given Name (first and middle [if any])				Family Name or Surname			
Karen Kulju				McKee			
Inventor's Signature	[signature]			**Date**	4/17/01		
Residence: City	Middletown	**State**	NJ NJ	**Country**	USA	**Citizenship**	US
Post Office Address	Merck & Co., Inc., P.O. Box 2000						
City	Rahway	**State**	NJ	**ZIP**	07065-0907		

5-00

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor						
Given Name (first and middle [if any])				Family Name or Surname			
Douglas				MacNeil			
Inventor's Signature	[signature]			**Date**	May 15, 2001		
Residence: City	Westfield	**State**	NJ NJ	**Country**	USA	**Citizenship**	US
Post Office Address	Merck & Co., Inc., P.O. Box 2000						
City	Rahway	**State**	NJ	**ZIP**	07065-0907		

Please type a plus sign (+) inside this box +

097[illegible]9495 .052501

[illegible]proved for use through 9/30/2000. OMB 651-0032
SUBSTITUTE for PTO/SB/02A (3-97), Declaration (Additional Inventors)

DECLARATION AND POWER OF ATTORNEY	**ADDITIONAL INVENTOR(S)** Supplemental Sheet

6-00

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor				
Given Name (first and middle [if any])			Family Name or Surname		
Andrew D.			Howard		
Inventor's Signature			**Date**	5/22/01	
Residence: City	Park Ridge	**State**	NJ NJ	**Country**	USA
Citizenship	US				
Post Office Address	Merck & Co., Inc., P.O. Box 2000				
City	Rahway	**State**	NJ	**ZIP**	07065-0907

7-00

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor				
Given Name (first and middle [if any])			Family Name or Surname		
Sheng-Shung			Pong		
Inventor's Signature			**Date**	21 May 2001	
Residence: City	Edison	**State**	NJ NJ	**Country**	USA
Citizenship	US				
Post Office Address	Merck & Co., Inc., P.O. Box 2000				
City	Rahway	**State**	NJ	**ZIP**	07065-0907

8-00

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor				
Given Name (first and middle [if any])			Family Name or Surname		
Roy G.			Smith		
Inventor's Signature			**Date**	16 May 2001	
Residence: City	Houston	**State**	TX TX	**Country**	USA
Citizenship	US				
Post Office Address	Merck & Co., Inc., P.O. Box 2000				
City	Rahway	**State**	NJ	**ZIP**	07065-0907

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor				
Given Name (first and middle [if any])			Family Name or Surname		
Inventor's Signature			**Date**		
Residence: City		**State**		**Country**	
Citizenship					
Post Office Address	Merck & Co., Inc., P O. Box 2000				
City	Rahway	**State**	NJ	**ZIP**	07065-0907

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Merck & Co., Inc.

(ii) TITLE OF INVENTION: CLONING AND IDENTIFICATION OF THE MOTILIN RECEPTOR

(iii) NUMBER OF SEQUENCES: 15

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Merck & Co., Inc.
(B) STREET: P.O. Box 2000, 126 E. Lincoln Ave.
(C) CITY: Rahway
(D) STATE: NJ
(E) COUNTRY: USA
(F) ZIP: 07065-0900

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: Windows
(D) SOFTWARE: FastSEQ for Windows Version 2.0b

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 60/089,098
(B) FILING DATE: 12-JUN-1998

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Giesser, Joanne M
(B) REGISTRATION NUMBER: 32,838
(C) REFERENCE/DOCKET NUMBER: 20251 PCT

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 732-594-3046
(B) TELEFAX: 732-594-4720
(C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3066 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
TTGAAATTAT CTGGTCACTG CCGGGCGCGG TGGCTCACGC CTGTAATCCC AGCACTTTGG     60
GAGGTCGAGG CGGGTGGACC ACCTGGGGTC AGGAGTTCGA GACCAGGCTG GCCAACATGG    120
CGAAACCCTG ACTACACAAA AAACACAAAA TTTAGCCGGG GCTTGGGCGC TCCTGTGCTC    180
CCAGCTACTC AGGAGGCTGA GGTGGGAGGA CTGCTTGAGC CTGGGAGGTC GAGGCTGCAG    240
TGAGCTGTGA TCGCGCCACT TAAACTCCAG CCTGGACGAC AGTGAGACCC TGTCTCAAGA    300
AGAAAAAAAG AAAGAAAGAA AGAAAAAAAG AAAAAAAAGA AATTATTTGG TCAATTATAT    360
GGTCAGCTCC CTCCACCACT CGCGAATTTA CAGAAGAGGA GAACTGGGCT GGGCGAGACC    420
AGGACTAGCC CAAGATTACA CAAGTTACTC GGTTGTAGAG CCAGGATTAG ACAGGAGAGG    480
CTCTAGATTC TGGTCTAGAC TCCCCTCCTA TTATTTAGCA TTATGGCTTC CTGAGGATTA    540
CCATGAGCCC TCCTCCACCG TCAAGCGGCA GCTACCAGCC ACCAGACCAG ATCCCTTCGA    600
AGGTGCCCGG AGTACCAGAC TGACAAAAGC GCCCGTACAG TGCTCAGTCC TGTAACCAAA    660
GCTGTCTAGG GTGCAGACAT CGCTCACCGG ACCGGGTAGG GCTCGTGCGC TAAGGGCGCC    720
GGGTATTCCA GTTAGTGGAG AGGGAAGCGC CCTGGAACTG CATGGGCCCG GGAGAGGGCG    780
CGGGAGCGGA GCATGGCCGG GCCGGGGCGG GCCGCGGCCG TGGGCGGAGA CTGCGCGCAG    840
CTAGCTCGGG AGCGCCTCGG AGCCCACCCC GCAGAGCCGC TTCTCGCGCC CCGCAGCGCA    900
GCGCAGCGCT CCGCCGTCTG ACCTGCCGCG CCCGCAGCGT GCGGGCTGGG AAAGGAGGCG    960
CTCACCGAGA GGGACCACGC GCCAGGCTCC CAGCCCGACC CGGGACGCGG CGGCCGCGCG   1020
GAGCACCCAT GGGCAGCCCC TGGAACGGCA GCGACGGCCC CGAGGGGGCG CGGGAGCCGC   1080
CGTGGCCCGC GCTGCCGCCT TGCGACGAGC GCCGCTGCTC GCCCTTTCCC CTGGGGGCGC   1140
TGGTGCCGGT GACCGCTGTG TGCCTGTGCC TGTTCGTCGT CGGGGTGAGC GGCAACGTGG   1200
TGACCGTGAT GCTGATCGGG CGCTACCGGG ACATGCGGAC CACCACCAAC TTGTACCTGG   1260
GCAGCATGGC CGTGTCCGAC CTACTCATCC TGCTCGGGCT GCCGTTCGAC CTGTACCGCC   1320
TCTGGCGCTC GCGGCCCTGG GTGTTCGGGC CGCTGCTCTG CCGCCTGTCC CTCTACGTGG   1380
GCGAGGGCTG CACCTACGCC ACGCTGCTGC ACATGACCGC GCTCAGCGTC GAGCGCTACC   1440
TGGCCATCTG CCGCCCGCTC CGCGCCCGCG TCTTGGTCAC CCGGCGCCGC GTCCGCGCGC   1500
TCATCGCTGT GCTCTGGGCC GTGGCGCTGC TCTCTGCCGG TCCCTTCTTG TTCCTGGTGG   1560
GCGTCGAGCA GGACCCCGGC ATCTCCGTAG TCCCGGGCCT CAATGGCACC GCGCGGATCG   1620
CCTCCTCGCC TCTCGCCTCG TCGCCGCCTC TCTGGCTCTC GCGGGCGCCA CCGCCGTCCC   1680
CGCCGTCGGG GCCCGAGACC GCGGAGGCCG CGGCGCTGTT CAGCCGCGAA TGCCGGCCGA   1740
GCCCCGCGCA GCTGGGCGCG CTGCGTGTCA TGCTGTGGGT CACCACCGCC TACTTCTTCC   1800
TGCCCTTTCT GTGCCTCAGC ATCCTCTACG GGCTCATCGG GCGGGAGCTG TGGAGCAGCC   1860
GGCGGCCGCT GCGAGGCCCG GCCGCCTCGG GGCGGGAGAG AGGCCACCGG CAGACCGTCC   1920
GCGTCCTGCG TAAGTGGAGC CGCCGTGGTT CCAAAGACGC CTGCCTGCAG TCCGCCCCGC   1980
CGGGGACCGC GCAAACGCTG GGTCCCCTTC CCCTGCTCGC CCAGCTCTGG GCGCCGCTTC   2040
CAGCTCCCTC CTATTTCGAT TCCAGCCTCC ACCCGCCGGT ACTTCCCATC CCCCGAGAAA   2100
ACCATGTCCT GTCCCCCAGG AGCTCTGGGG GACCCCAGGG CGCTTTGAGG GTGGGATCCC   2160
CGGATCCGAT TCAGTAACCA GCAGTGCTTT TCCAGAGCCT CTGAGACCAG AAAGGAGAGT   2220
TGGTAATTCT TAATCCAACC ACCTGTTAGA TGCCACAAAT GAGGAGTCCT CACAGTGCTC   2280
TTGAGAAGAC GAGGGAGATT TCATTAAGCT AAAATTTTTT ATTTAATGTT AAGTGATGCT   2340
GAAGGCTAAA GTAAACCTTG CTCGTATCAA AAAGTAAAGA TTGTGCAGAC CTGTTGTAGA   2400
ATTCTTTTCA ACAGAGAACA GAAAACTTGT CTCCGAAGTG GGTTTGTGGA AGGAAGCCTG   2460
CCAAGGCGGC TTGTTCAGAG AAATTGCTCC TTCTGGTTTA TGTCCAGCCT TGATAACACA   2520
TATGGGAGCC TACTATGCAG TTTTAAAGCA AGTATCCATG CAGCCTGCAG CCTGGTCATT   2580
TTTTCTGGGG TGAGGATCTG CCTAGGTAGA AGTTTTCTCT AATTTATTTT GCTGTTACTT   2640
GTTATTGCAG ATGGTTCCTT GTCGGGGTGG GGGGTTTATT TGCTTCCCAA TGCTTTTGTT   2700
AATCCCGGTG CTGTGTCTTA TGTTGCAGTG GTGGTGGTTC TGGCATTTAT AATTTGCTGG   2760
TTGCCCTTCC ACGTTGGCAG AATCATTTAC ATAAACACGG AAGATTCGCG GATGATGTAC   2820
TTCTCTCAGT ACTTTAACAT CGTCGCTCTG CAACTTTTCT ATCTGAGCGC ATCTATCAAC   2880
CCAATCCTCT ACAACCTCAT TTCAAAGAAG TACAGAGCGG CGGCCTTTAA ACTGCTGCTC   2940
GCAAGGAAGT CCAGGCCGAG AGGCTTCCAC AGAAGCAGGG ACACTGCGGG GGAAGTTGCA   3000
GGGGACACTG GAGGAGACAC GGTGGGCTAC ACCGAGACAA GCGCTAACGT GAAGACGATG   3060
GGATAA                                                              3066
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1239 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
ATGGGCAGCC CCTGGAACGG CAGCGACGGC CCCGAGGGGG CGCGGGAGCC GCCGTGGCCC     60
GCGCTGCCGC CTTGCGACGA GCGCCGCTGC TCGCCCTTTC CCCTGGGGGC GCTGGTGCCG    120
GTGACCGCTG TGTGCCTGTG CCTGTTCGTC GTCGGGGTGA GCGGCAACGT GGTGACCGTG    180
ATGCTGATCG GGCGCTACCG GGACATGCGG ACCACCACCA ACTTGTACCT GGGCAGCATG    240
GCCGTGTCCG ACCTACTCAT CCTGCTCGGG CTGCCGTTCG ACCTGTACCG CCTCTGGCGC    300
TCGCGGCCCT GGGTGTTCGG GCCGCTGCTC TGCCGCCTGT CCCTCTACGT GGGCGAGGGC    360
TGCACCTACG CCACGCTGCT GCACATGACC GCGCTCAGCG TCGAGCGCTA CCTGGCCATC    420
TGCCGCCCGC TCCGCGCCCG CGTCTTGGTC ACCCGGCGCC GCGTCCGCGC GCTCATCGCT    480
GTGCTCTGGG CCGTGGCGCT GCTCTCTGCC GGTCCCTTCT TGTTCCTGGT GGGCGTCGAG    540
CAGGACCCCG GCATCTCCGT AGTCCCGGGC CTCAATGGCA CCGCGCGGAT CGCCTCCTCG    600
CCTCTCGCCT CGTCGCCGCC TCTCTGGCTC TCGCGGGCGC CACCGCCGTC CCCGCCGTCG    660
GGGCCCGAGA CCGCGGAGGC CGCGGCGCTG TTCAGCCGCG AATGCCGGCC GAGCCCCGCG    720
CAGCTGGGCG CGCTGCGTGT CATGCTGTGG GTCACCACCG CCTACTTCTT CCTGCCCTTT    780
CTGTGCCTCA GCATCCTCTA CGGGCTCATC GGGCGGGAGC TGTGGAGCAG CCGGCGGCCG    840
CTGCGAGGCC CGGCCGCCTC GGGGCGGGAG AGAGGCCACC GGCAGACCGT CCGCGTCCTG    900
CTGGTGGTGG TTCTGGCATT TATAATTTGC TGGTTGCCCT TCCACGTTGG CAGAATCATT    960
TACATAAACA CGGAAGATTC GCGGATGATG TACTTCTCTC AGTACTTTAA CATCGTCGCT   1020
CTGCAACTTT TCTATCTGAG CGCATCTATC AACCCAATCC TCTACAACCT CATTTCAAAG   1080
AAGTACAGAG CGGCGGCCTT TAAACTGCTG CTCGCAAGGA AGTCCAGGCC GAGAGGCTTC   1140
CACAGAAGCA GGGACACTGC GGGGGAAGTT GCAGGGGACA CTGGAGGAGA CACGGTGGGC   1200
TACACCGAGA CAAGCGCTAA CGTGAAGACG ATGGGATAA                          1239
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 412 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Met Gly Ser Pro Trp Asn Gly Ser Asp Gly Pro Glu Gly Ala Arg Glu
 1               5                  10                  15
Pro Pro Trp Pro Ala Leu Pro Pro Cys Asp Glu Arg Arg Cys Ser Pro
            20                  25                  30
Phe Pro Leu Gly Ala Leu Val Pro Val Thr Ala Val Cys Leu Cys Leu
        35                  40                  45
Phe Val Val Gly Val Ser Gly Asn Val Val Thr Val Met Leu Ile Gly
    50                  55                  60
Arg Tyr Arg Asp Met Arg Thr Thr Thr Asn Leu Tyr Leu Gly Ser Met
65                  70                  75                  80
```

```
Ala Val Ser Asp Leu Leu Ile Leu Leu Gly Leu Pro Phe Asp Leu Tyr
                85                  90                  95
Arg Leu Trp Arg Ser Arg Pro Trp Val Phe Gly Pro Leu Leu Cys Arg
            100                 105                 110
Leu Ser Leu Tyr Val Gly Glu Gly Cys Thr Tyr Ala Thr Leu Leu His
        115                 120                 125
Met Thr Ala Leu Ser Val Glu Arg Tyr Leu Ala Ile Cys Arg Pro Leu
    130                 135                 140
Arg Ala Arg Val Leu Val Thr Arg Arg Arg Val Arg Ala Leu Ile Ala
145                 150                 155                 160
Val Leu Trp Ala Val Ala Leu Leu Ser Ala Gly Pro Phe Leu Phe Leu
                165                 170                 175
Val Gly Val Glu Gln Asp Pro Gly Ile Ser Val Val Pro Gly Leu Asn
            180                 185                 190
Gly Thr Ala Arg Ile Ala Ser Ser Pro Leu Ala Ser Ser Pro Pro Leu
        195                 200                 205
Trp Leu Ser Arg Ala Pro Pro Pro Ser Pro Pro Ser Gly Pro Glu Thr
    210                 215                 220
Ala Glu Ala Ala Ala Leu Phe Ser Arg Glu Cys Arg Pro Ser Pro Ala
225                 230                 235                 240
Gln Leu Gly Ala Leu Arg Val Met Leu Trp Val Thr Thr Ala Tyr Phe
                245                 250                 255
Phe Leu Pro Phe Leu Cys Leu Ser Ile Leu Tyr Gly Leu Ile Gly Arg
            260                 265                 270
Glu Leu Trp Ser Ser Arg Arg Pro Leu Arg Gly Pro Ala Ala Ser Gly
        275                 280                 285
Arg Glu Arg Gly His Arg Gln Thr Val Arg Val Leu Leu Val Val Val
    290                 295                 300
Leu Ala Phe Ile Ile Cys Trp Leu Pro Phe His Val Gly Arg Ile Ile
305                 310                 315                 320
Tyr Ile Asn Thr Glu Asp Ser Arg Met Met Tyr Phe Ser Gln Tyr Phe
                325                 330                 335
Asn Ile Val Ala Leu Gln Leu Phe Tyr Leu Ser Ala Ser Ile Asn Pro
            340                 345                 350
Ile Leu Tyr Asn Leu Ile Ser Lys Lys Tyr Arg Ala Ala Ala Phe Lys
        355                 360                 365
Leu Leu Leu Ala Arg Lys Ser Arg Pro Arg Gly Phe His Arg Ser Arg
    370                 375                 380
Asp Thr Ala Gly Glu Val Ala Gly Asp Thr Gly Gly Asp Thr Val Gly
385                 390                 395                 400
Tyr Thr Glu Thr Ser Ala Asn Val Lys Thr Met Gly
                405                 410
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1390 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
ATGGGCAGCC CCTGGAACGG CAGCGACGGC CCCGAGGGGG CGCGGGAGCC GCCGTGGCCC        60
```

GCGCTGCCGC CTTGCGACGA GCGCCGCTGC TCGCCCTTTC CCCTGGGGGC GCTGGTGCCG 120
GTGACCGCTG TGTGCCTGTG CCTGTTCGTC GTCGGGGTGA GCGGCAACGT GGTGACCGTG 180
ATGCTGATCG GGCGCTACCG GGACATGCGG ACCACCACCA ACTTGTACCT GGGCAGCATG 240
GCCGTGTCCG ACCTACTCAT CCTGCTCGGG CTGCCGTTCG ACCTGTACCG CCTCTGGCGC 300
TCGCGGCCCT GGGTGTTCGG GCCGCTGCTC TGCCGCCTGT CCCTCTACGT GGGCGAGGGC 360
TGCACCTACG CCACGCTGCT GCACATGACC GCGCTCAGCG TCGAGCGCTA CCTGGCCATC 420
TGCCGCCCGC TCCGCGCCCG CGTCTTGGTC ACCCGGCGCC GCGTCCGCGC GCTCATCGCT 480
GTGCTCTGGG CCGTGGCGCT GCTCTCTGCC GGTCCCTTCT TGTTCCTGGT GGGCGTCGAG 540
CAGGACCCCG GCATCTCCGT AGTCCCGGGC CTCAATGGCA CCGCGCGGAT CGCCTCCTCG 600
CCTCTCGCCT CGTCGCCGCC TCTCTGGCTC TCGCGGGCGC CACCGCCGTC CCCGCCGTCG 660
GGGCCCGAGA CCGCGGAGGC CGCGGCGCTG TTCAGCCGCG AATGCCGGCC GAGCCCCGCG 720
CAGCTGGGCG CGCTGCGTGT CATGCTGTGG GTCACCACCG CCTACTTCTT CCTGCCCTTT 780
CTGTGCCTCA GCATCCTCTA CGGGCTCATC GGGCGGGAGC TGTGGAGCAG CCGGCGGCCG 840
CTGCGAGGCC CGGCCGCCTC GGGGCGGGAG AGAGGCCACC GGCAGACCGT CCGCGTCCTG 900
CGTAAGTGGA GCCGCCGTGG TTCCAAAGAC GCCTGCCTGC AGTCCGCCCC GCCGGGGACC 960
GCGCAAACGC TGGGTCCCCT TCCCCTGCTC GCCCAGCTCT GGGCGCCGCT TCCAGCTCCC 1020
TTTCCTATTT CGATTCCAGC CTCCACCCGC CGTGGTGGTG GTTCTGGCAT TTATAATTTG 1080
CTGGTTGCCC TTCCACGTTG GCAGAATCAT TTACATAAAC ACGGAAGATT CGCGGATGAT 1140
GTACTTCTCT CAGTACTTTA ACATCGTCGC TCTGCAACTT TTCTATCTGA GCGCATCTAT 1200
CAACCCAATC CTCTACAACC TCATTTCAAA GAAGTACAGA GCGGCGGCCT TTAAACTGCT 1260
GCTCGCAAGG AAGTCCAGGC CGAGAGGCTT CCACAGAAGC AGGGACACTG CGGGGGAAGT 1320
TGCAGGGGAC ACTGGAGGAG ACACGGTGGG CTACACCGAG ACAAGCGCTA ACGTGAAGAC 1380
GATGGGATAA 1390

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 386 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met Gly Ser Pro Trp Asn Gly Ser Asp Gly Pro Glu Gly Ala Arg Glu
1 5 10 15
Pro Pro Trp Pro Ala Leu Pro Pro Cys Asp Glu Arg Arg Cys Ser Pro
20 25 30
Phe Pro Leu Gly Ala Leu Val Pro Val Thr Ala Val Cys Leu Cys Leu
35 40 45
Phe Val Val Gly Val Ser Gly Asn Val Val Thr Val Met Leu Ile Gly
50 55 60
Arg Tyr Arg Asp Met Arg Thr Thr Thr Asn Leu Tyr Leu Gly Ser Met
65 70 75 80
Ala Val Ser Asp Leu Leu Ile Leu Leu Gly Leu Pro Phe Asp Leu Tyr
85 90 95
Arg Leu Trp Arg Ser Arg Pro Trp Val Phe Gly Pro Leu Leu Cys Arg
100 105 110
Leu Ser Leu Tyr Val Gly Glu Gly Cys Thr Tyr Ala Thr Leu Leu His
115 120 125
Met Thr Ala Leu Ser Val Glu Arg Tyr Leu Ala Ile Cys Arg Pro Leu
130 135 140
Arg Ala Arg Val Leu Val Thr Arg Arg Arg Val Arg Ala Leu Ile Ala
145 150 155 160

```
Val Leu Trp Ala Val Ala Leu Leu Ser Ala Gly Pro Phe Leu Phe Leu
                165                 170                 175
Val Gly Val Glu Gln Asp Pro Gly Ile Ser Val Val Pro Gly Leu Asn
            180                 185                 190
Gly Thr Ala Arg Ile Ala Ser Ser Pro Leu Ala Ser Ser Pro Pro Leu
        195                 200                 205
Trp Leu Ser Arg Ala Pro Pro Pro Ser Pro Pro Ser Gly Pro Glu Thr
    210                 215                 220
Ala Glu Ala Ala Ala Leu Phe Ser Arg Glu Cys Arg Pro Ser Pro Ala
225                 230                 235                 240
Gln Leu Gly Ala Leu Arg Val Met Leu Trp Val Thr Thr Ala Tyr Phe
                245                 250                 255
Phe Leu Pro Phe Leu Cys Leu Ser Ile Leu Tyr Gly Leu Ile Gly Arg
            260                 265                 270
Glu Leu Trp Ser Ser Arg Arg Pro Leu Arg Gly Pro Ala Ala Ser Gly
        275                 280                 285
Arg Glu Arg Gly His Arg Gln Thr Val Arg Val Leu Arg Lys Trp Ser
    290                 295                 300
Arg Arg Gly Ser Lys Asp Ala Cys Leu Gln Ser Ala Pro Pro Gly Thr
305                 310                 315                 320
Ala Gln Thr Leu Gly Pro Leu Pro Leu Leu Ala Gln Leu Trp Ala Pro
                325                 330                 335
Leu Pro Ala Pro Phe Pro Ile Ser Ile Pro Ala Ser Thr Arg Arg Gly
            340                 345                 350
Gly Gly Ser Gly Ile Tyr Asn Leu Leu Val Ala Leu Pro Arg Trp Gln
        355                 360                 365
Asn His Leu His Lys His Gly Arg Phe Ala Asp Asp Val Leu Leu Ser
    370                 375                 380
Val Leu
385
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1092 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
ATGCCCTGGA CCAGACCCCA GGTGGACCTC CATGCTGCTG CAGCAGAGAC CATGGACCAG    60
TACACCACGG ACGACCACCA CTACGAGGGC TCCCTCTTCC CCGCGTCCAC CCTCATCCCC   120
GTCACGGTCA TCTGCATCCT CATCTTCGTG GTCGGCGTGA CCGGCAACAC CATGACCATC   180
CTCATCATCC AGTACTTCAA GGACATGAAG ACCACCACCA ACCTGTACCT GTCCAGCATG   240
GCCGTGTCCG ACCTCGTCAT CTTCCTCTGC CTGCCCTTCG ACCTGTACCG CCTGTGGAAG   300
TACGTGCCGT GGCTGTTCGG CGAGGCCGTG TGCCGCCTCT ACCACTACAT CTTCGAAGGC   360
TGCACGTCGG CCACCATCCT CCACATCACG GCCCTGAGCA TCGAGCGCTA CCTGGCCATC   420
AGCTTCCCCC TCAGGAGCAA GGTGATGGTG ACCAGGAGAA GGGTCCAGTA CATCATCCTG   480
GCCCTGTGGT GCTTCGCCCT GGTGTCGGCC GCTCCCACGC TCTTCCTGGT CGGGGTGGAG   540
TACGACAACG AGACGCACCC CGACTACAAC ACGGGCCAGT GCAAGCACAC GGGCTACGCC   600
ATCAGCTCGG GGCAGCTGCA CATCATGATC TGGGTGTCCA CCACCTACTT CTTCTGCCCG   660
ATGCTGTGTC TCCTCTTCCT CTACGGCTCC ATCGGGTGCA AGCTGTGGAA GAGCAAGAAC   720
GACCTGCAGG GCCCGTGCGC CCTGGCCCGC GAGAGGTCGC ACAGGCAAAC GGTGAAGATC   780
```

```
CTGGTGGTGG TGGTGCTGGC CTTCATCATC TGCTGGCTGC CCTACCACAT CGGCAGGAAC     840
CTGTTCGCCC AGGTGGACGA CTACGACACG GCCATGCTCA GCCAGAATTT CAACATGGCC     900
TCCATGGTGC TCTGCTACCT CAGCGCCTCC ATCAACCCCG TCGTCTACAA CCTGATGTCG     960
AGGAAGTACC GGGCCGCCGC CAAGCGCCTC TTCCTGCTCC ACCAGAGACC CAAGCCGGCC    1020
CACCGGGGGC AGGGGCAGTT TTGCATGATC GGCCACAGCC CCACCCTGGA CGAGAGCCTG    1080
ACGGGGGTGT GA                                                        1092
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 363 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
Met Pro Trp Thr Arg Pro Gln Val Asp Leu His Ala Ala Ala Ala Glu
 1               5                  10                  15
Thr Met Asp Gln Tyr Thr Thr Asp Asp His His Tyr Glu Gly Ser Leu
            20                  25                  30
Phe Pro Ala Ser Thr Leu Ile Pro Val Thr Val Ile Cys Ile Leu Ile
        35                  40                  45
Phe Val Val Gly Val Thr Gly Asn Thr Met Thr Ile Leu Ile Ile Gln
    50                  55                  60
Tyr Phe Lys Asp Met Lys Thr Thr Thr Asn Leu Tyr Leu Ser Ser Met
65                  70                  75                  80
Ala Val Ser Asp Leu Val Ile Phe Leu Cys Leu Pro Phe Asp Leu Tyr
                85                  90                  95
Arg Leu Trp Lys Tyr Val Pro Trp Leu Phe Gly Glu Ala Val Cys Arg
            100                 105                 110
Leu Tyr His Tyr Ile Phe Glu Gly Cys Thr Ser Ala Thr Ile Leu His
        115                 120                 125
Ile Thr Ala Leu Ser Ile Glu Arg Tyr Leu Ala Ile Ser Phe Pro Leu
    130                 135                 140
Arg Ser Lys Val Met Val Thr Arg Arg Arg Val Gln Tyr Ile Ile Leu
145                 150                 155                 160
Ala Leu Trp Cys Phe Ala Leu Val Ser Ala Ala Pro Thr Leu Phe Leu
                165                 170                 175
Val Gly Val Glu Tyr Asp Asn Glu Thr His Pro Asp Tyr Asn Thr Gly
            180                 185                 190
Gln Cys Lys His Thr Gly Tyr Ala Ile Ser Ser Gly Gln Leu His Ile
        195                 200                 205
Met Ile Trp Val Ser Thr Thr Tyr Phe Phe Cys Pro Met Leu Cys Leu
    210                 215                 220
Leu Phe Leu Tyr Gly Ser Ile Gly Cys Lys Leu Trp Lys Ser Lys Asn
225                 230                 235                 240
Asp Leu Gln Gly Pro Cys Ala Leu Ala Arg Glu Arg Ser His Arg Gln
                245                 250                 255
Thr Val Lys Ile Leu Val Val Val Val Leu Ala Phe Ile Ile Cys Trp
            260                 265                 270
Leu Pro Tyr His Ile Gly Arg Asn Leu Phe Ala Gln Val Asp Asp Tyr
        275                 280                 285
```

```
Asp Thr Ala Met Leu Ser Gln Asn Phe Asn Met Ala Ser Met Val Leu
    290                 295                 300
Cys Tyr Leu Ser Ala Ser Ile Asn Pro Val Val Tyr Asn Leu Met Ser
305                 310                 315                 320
Arg Lys Tyr Arg Ala Ala Ala Lys Arg Leu Phe Leu Leu His Gln Arg
                325                 330                 335
Pro Lys Pro Ala His Arg Gly Gln Gly Gln Phe Cys Met Ile Gly His
            340                 345                 350
Ser Pro Thr Leu Asp Glu Ser Leu Thr Gly Val
        355                 360
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CCATCCTAAT ACGACTCACT ATAGGGC 27

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TTATCCCATC GTCTTCACGT TAGCGCTTGT CTC 33

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CTGCCCTTTC TGTGCCTCAG CATCCTCTAC 30

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 900 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
ATGGGCAGCC CCTGGAACGG CAGCGACGGC CCCGAGGGGG CGCGGGAGCC GCCGTGGCCC    60
GCGCTGCCGC CTTGCGACGA GCGCCGCTGC TCGCCCTTTC CCCTGGGGGC GCTGGTGCCG   120
GTGACCGCTG TGTGCCTGTG CCTGTTCGTC GTCGGGGTGA GCGGCAACGT GGTGACCGTG   180
ATGCTGATCG GGCGCTACCG GGACATGCGG ACCACCACCA ACTTGTACCT GGGCAGCATG   240
GCCGTGTCCG ACCTACTCAT CCTGCTCGGG CTGCCGTTCG ACCTGTACCG CCTCTGGCGC   300
TCGCGGCCCT GGGTGTTCGG GCCGCTGCTC TGCCGCCTGT CCCTCTACGT GGGCGAGGGC   360
TGCACCTACG CCACGCTGCT GCACATGACC GCGCTCAGCG TCGAGCGCTA CCTGGCCATC   420
TGCCGCCCGC TCCGCGCCCG CGTCTTGGTC ACCCGGCGCC GCGTCCGCGC GCTCATCGCT   480
GTGCTCTGGG CCGTGGCGCT GCTCTCTGCC GGTCCCTTCT TGTTCCTGGT GGGCGTCGAG   540
CAGGACCCCG GCATCTCCGT AGTCCCGGGC CTCAATGGCA CCGCGCGGAT CGCCTCCTCG   600
CCTCTCGCCT CGTCGCCGCC TCTCTGGCTC TCGCGGGCGC CACCGCCGTC CCCGCCGTCG   660
GGGCCCGAGA CCGCGGAGGC CGCGGCGCTG TTCAGCCGCG AATGCCGGCC GAGCCCCGCG   720
CAGCTGGGCG CGCTGCGTGT CATGCTGTGG GTCACCACCG CCTACTTCTT CCTGCCCTTT   780
CTGTGCCTCA GCATCCTCTA CGGGCTCATC GGGCGGGAGC TGTGGAGCAG CCGGCGGCCG   840
CTGCGAGGCC CGGCCGCCTC GGGGCGGGAG AGAGGCCACC GGCAGACCGT CCGCGTCCTG   900
```

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 300 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
Met Gly Ser Pro Trp Asn Gly Ser Asp Gly Pro Glu Gly Ala Arg Glu
 1               5                  10                  15
Pro Pro Trp Pro Ala Leu Pro Pro Cys Asp Glu Arg Arg Cys Ser Pro
            20                  25                  30
Phe Pro Leu Gly Ala Leu Val Pro Val Thr Ala Val Cys Leu Cys Leu
        35                  40                  45
Phe Val Val Gly Val Ser Gly Asn Val Val Thr Val Met Leu Ile Gly
    50                  55                  60
Arg Tyr Arg Asp Met Arg Thr Thr Thr Asn Leu Tyr Leu Gly Ser Met
65                  70                  75                  80
Ala Val Ser Asp Leu Leu Ile Leu Leu Gly Leu Pro Phe Asp Leu Tyr
                85                  90                  95
Arg Leu Trp Arg Ser Arg Pro Trp Val Phe Gly Pro Leu Leu Cys Arg
            100                 105                 110
Leu Ser Leu Tyr Val Gly Glu Gly Cys Thr Tyr Ala Thr Leu Leu His
        115                 120                 125
Met Thr Ala Leu Ser Val Glu Arg Tyr Leu Ala Ile Cys Arg Pro Leu
    130                 135                 140
```

```
Arg Ala Arg Val Leu Val Thr Arg Arg Arg Val Arg Ala Leu Ile Ala
145                 150                 155                 160
Val Leu Trp Ala Val Ala Leu Leu Ser Ala Gly Pro Phe Leu Phe Leu
                165                 170                 175
Val Gly Val Glu Gln Asp Pro Gly Ile Ser Val Val Pro Gly Leu Asn
            180                 185                 190
Gly Thr Ala Arg Ile Ala Ser Ser Pro Leu Ala Ser Ser Pro Pro Leu
        195                 200                 205
Trp Leu Ser Arg Ala Pro Pro Pro Ser Pro Pro Ser Gly Pro Glu Thr
    210                 215                 220
Ala Glu Ala Ala Ala Leu Phe Ser Arg Glu Cys Arg Pro Ser Pro Ala
225                 230                 235                 240
Gln Leu Gly Ala Leu Arg Val Met Leu Trp Val Thr Thr Ala Tyr Phe
                245                 250                 255
Phe Leu Pro Phe Leu Cys Leu Ser Ile Leu Tyr Gly Leu Ile Gly Arg
            260                 265                 270
Glu Leu Trp Ser Ser Arg Arg Pro Leu Arg Gly Pro Ala Ala Ser Gly
        275                 280                 285
Arg Glu Arg Gly His Arg Gln Thr Val Arg Val Leu
    290                 295                 300
```

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 154 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
CGTAAGTGGA GCCGCCGTGG TTCCAAAGAC GCCTGCCTGC AGTCCGCCCC GCCGGGGACC     60
GCGCAAACGC TGGGTCCCCT TCCCCTGCTC GCCCAGCTCT GGGCGCCGCT TCCAGCTCCC    120
TTTCCTATTT CGATTCCAGC CTCCACCCGC CGGT                                154
```

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 602 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
AGCTGGTGGT GGTTCTGGCA TTTATAATTT GCTGGTTGCC CTTCCACGTT GGCAGAATCA     60
TTTACATAAA CACGGAAGAT TCGCGGATGA TGTACTTCTC TCAGTACTTT AACATCGTCG    120
CTCTGCAACT TTTCTATCTG AGCGCATCTA TCAACCCAAT CCTCTACAAC CTCATTTCAA    180
AGAAGTACAG AGCGGCGGCC TTTAAACTGC TGCTCGCAAG GAAGTCCAGG CCGAGAGGCT    240
TCCACAGAAG CAGGGACACT GCGGGGGAAG TTGCAGGGGA CACTGGAGGA GACACGGTGG    300
GCTACACCGA GACAAGCGCT AACGTGAAGA CGATGGGATA ACGTAAGTGG AGCCGCCGTG    360
GTTCCAAAGA CGCCTGCCTG CAGTCCGCCC CGCCGGGGAC CGCGCAAACG CTGGGTCCCC    420
```

```
TTCCCCTGCT CGCCCAGCTC TGGGCGCCGC TTCCAGCTCC CTTTCCTATT TCGATTCCAG    480
CCTCCACCCG CCGTGGTGGT GGTTCTGGCA TTTATAATTT GCTGGTTGCC CTTCCACGTT    540
GGCAGAATCA TTTACATAAA CACGGAAGAT TCGCGGATGA TGTACTTCTC TCAGTACTTT    600
AA                                                                   602
```

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 198 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

```
Leu Val Val Val Leu Ala Phe Ile Ile Cys Trp Leu Pro Phe His Val
 1               5                  10                  15
Gly Arg Ile Ile Tyr Ile Asn Thr Glu Asp Ser Arg Met Met Tyr Phe
            20                  25                  30
Ser Gln Tyr Phe Asn Ile Val Ala Leu Gln Leu Phe Tyr Leu Ser Ala
        35                  40                  45
Ser Ile Asn Pro Ile Leu Tyr Asn Leu Ile Ser Lys Lys Tyr Arg Ala
    50                  55                  60
Ala Ala Phe Lys Leu Leu Leu Ala Arg Lys Ser Arg Pro Arg Gly Phe
65                  70                  75                  80
His Arg Ser Arg Asp Thr Ala Gly Glu Val Ala Gly Asp Thr Gly Gly
                85                  90                  95
Asp Thr Val Gly Tyr Thr Glu Thr Ser Ala Asn Val Lys Thr Met Gly
            100                 105                 110
Arg Lys Trp Ser Arg Arg Gly Ser Lys Asp Ala Cys Leu Gln Ser Ala
        115                 120                 125
Pro Pro Gly Thr Ala Gln Thr Leu Gly Pro Leu Pro Leu Leu Ala Gln
    130                 135                 140
Leu Trp Ala Pro Leu Pro Ala Pro Phe Pro Ile Ser Ile Pro Ala Ser
145                 150                 155                 160
Thr Arg Arg Gly Gly Gly Ser Gly Ile Tyr Asn Leu Leu Val Ala Leu
                165                 170                 175
Pro Arg Trp Gln Asn His Leu His Lys His Gly Arg Phe Ala Asp Asp
            180                 185                 190
Val Leu Leu Ser Val Leu
        195
```